RESEARCH AND REPORTS

ON FROZEN HUMAN CELL CULTURES AS A TIME-INDEPENDENT RADIATION DOSIMETER

by

ROBERT H. FETNER

Project A-572

Engineering Experiment Station
Georgia Institute of Technology
Atlanta, 1961-63

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Formal Progress Report.

No. 1. December 31, 1961.

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No. 2. February 1, 1962--April 30, 1962.
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No. 4. August 1, 1962--October 31, 1962.
No. 5. November 1, 1962--January 31, 1963.

Final Report.

June 30, 1963.

FORMAL PROGRESS REPORT NO. 1

PROJECT NO. A-572

RESEARCH AND REPORTS ON FROZEN HUMAN CELL CULTURES AS A TIME-INDEPENDENT RADIATION DOSIMETER

By

ROBERT H. FETNER

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CONTRACT NO. AF 41(657)-408

SCHOOL OF AVIATION MEDICINE

UNITED STATES AIR FORCE
RANDOLPH AIR FORCE BASE, TEXAS
R&D PROJECT NO. 7757

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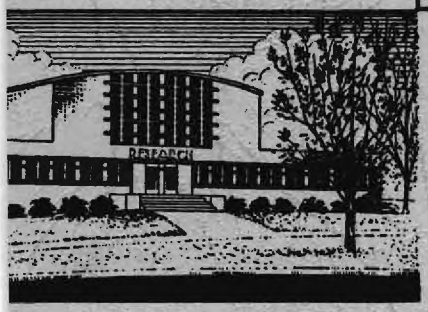
DECEMBER 31, 1961



Engineering Experiment Station
Georgia Institute of Technology
Atlanta, Georgia

ENGINEERING EXPERIMENT STATION
of the Georgia Institute of Technology
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FORMAL PROGRESS REPORT NO. 1

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DECEMBER 31, 1961

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I. ABSTRACT

Cell cultures have been irradiated in the frozen and unfrozen state and slide preparations have been made at 12-hour intervals for 60 hours. The experiments with unfrozen cells have involved doses of 50, 100, 200, 400, 800, and 1,000 r, and the experiments with frozen cultures were given 50, 100, 200, 400, 800, 1,200, 1,600, 2,000, 3,000, 4,000, and 5,000 r. The higher dosages with the frozen group have been dictated by a preliminary analysis which indicates a reduction of biological effectiveness of radiation to cells in the frozen state. Concomitantly with the irradiation of frozen cell cultures, irradiations were performed on a frozen and unfrozen chemical dosimeter. The Fricke ferric sulfate dosimeter has been selected and irradiation has been performed with ampuls of the solution at room temperature and submerged in liquid nitrogen. The preliminary data suggest that irradiation of ferrous ammonium sulfate solution in liquid nitrogen results in approximately one-fourth the yield of ferric ions that is produced by the same irradiation at room temperature; however, the yield of ferric ion as a function of dose did not show as good a fit to a straight line in the frozen state as is characteristic of this dosimeter in the unfrozen condition. Additional quantitation is being obtained.

II. STATEMENT OF PROGRESS

Experimental Work

Considerable progress has been made in the investigations for the development of an effective radiation dosimeter using frozen human cell cultures.

The KB cell line used in these investigations is maintained routinely on Eagles' media with 10 per cent pooled human serum. Cells to be irradiated in the frozen state are harvested from cultures in the logrithmetic phase of growth by scrapping from the glass surface and are suspended in a media composed of 50 per cent Hanks' saline, 40 per cent pooled human serum and 10 per cent glycerol. The cells are sealed in glass ampuls and frozen at a constant rate of 1° per minute until they reach -50° C. At this time they are then transferred to liquid nitrogen and placed in a liquid nitrogen refrigerator. Cells are irradiated submerged in liquid nitrogen in an open top vacuum flask. After irradiation cells are rapidly thawed, centrifuged 5 minutes at 500 rpm, resuspended in a complete growth media and incubated at 35° C. Every 12 hours, for 60 hours, the cultures are agitated to dislodge the metaphase cells and then the media which contains the cells diluted to 20 per cent concentration and kept at constant temperature for 20 minutes. To this hypotonic media enough acetic acid is added to bring the concentration of acid to 20 per cent. The cells are then concentrated by centrifugation, the fixative decanted and propionic acid-orcein dye added. Cells are then recentrifuged and the excess dye decanted from the cell pellet. This is placed on a slide, a cover slip added, and the cells pressed out manually until a satisfactory preparation is obtained. Slides are then made permanent with the dry-ice technic. Such preparations give well flattened and spread

metaphase figures suitable for detailed chromosome analysis. Cells were harvested in this manner at 12-hour intervals for a period of 60 hours. In this way it will be possible to determine if there is a peak production of the type of aberrations to be analyzed and also to determine how long this peak persists. This will permit a maximum figure to be obtained for aberrations as a function of dose and will prevent errors from being introduced as a result of such physiological processes as mitotic inhibition which may be more prevalent as the doses become greater. In those experiments where irradiations were performed on unfrozen cell cultures these cells were harvested by scrapping, placed in the ampuls, irradiated under the X-ray machine and then treated exactly as were the frozen cultures. Duplicate sets of experiments have been performed on both frozen and unfrozen cell cultures. The experiments with unfrozen cells have involved doses of 50, 100, 200, 400, 600, 800 and 1,000 r and the experiments with frozen cultures have been given doses of 50, 100, 200, 400, 800, 1,200, 1,600, 2,000, 3,000, 4,000 and 5,000 r. The higher dosages with the frozen group has been dictated by a preliminary analyses which indicate a reduction of biological effectiveness of radiation to cells in the frozen state of approximately the same magnitude as has been exhibited in the chemical dosimeter. Thus, 180 separate experiments have been performed, 70 in the unfrozen group (controlled group) and 110 in the frozen cell culture experiments. These slides are now being analyzed.

Concomitantly with the irradiation of frozen cell cultures irradiations were performed on a frozen and unfrozen chemical dosimeter. This appeared to be a reasonable approach for the determination of the relative dosages involved in the frozen and unfrozen state. The Fricke ferrous sulfate dosimeter was selected because of the reliability and relatively large amount of quantitative

work which has been performed with this technic[1].

Ten-milliliter ampuls were filled with ferrous ammonium sulfate solutions and heat sealed. Some of the ampuls were exposed unfrozen and some were frozen and exposed to the radiation submerged in liquid nitrogen in open top vacuum containers. After irradiation, ampuls were thawed and the concentration of ferric ion measured in the spectrophotometer. The absorbence (optical density) was determined at 305 mμ as has been the standard in the past. An analysis was also made at 224 mμ, as has been suggested by Scharf and Lee[2], who claim a two-fold increase in sensitivity at this wave length due to the high molar extinction coefficient, which is 4565 liters per mole centimeter at 224 mμ as compared to 2196 liters per mole centimeter at 304 mμ at 25° C. An additional advantage of the 224 mμ analysis is the much lower temperature dependence, which is 0.1 per cent at 224 mμ as compared to 0.7 per cent per °C at 304 mμ. Figure 1 shows an actual analysis at the two different wave lengths. A series of experiments was run in which the Fricke dosimeter was irradiated in both the frozen and unfrozen state. The results are presented in Figure 2. Irradiation of the ferrous ammonium sulfate solution in liquid nitrogen results in approximately one-fourth the yield of ferric ions that is produced by irradiation at room temperature. The yield, as a function of

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1. Weiss, J., A. O. Allen and H. A. Schwarz. Int. Conf. Peaceful Uses of Atomic Energy (1955)

2. Scharf, K. and R. M. Lee. Rad. Research 14, 498 (1961)

dose, did not show as good a fit to a straight line in the frozen state as is characteristic of this dosimeter in the unfrozen condition, so additional quantitation will be needed.

III. FUTURE PLANS

Slide preparations are being analyzed and when this is completed the production of chromosome breakage as a function of radiation dose will have been determined over a wide dose range, both for cells irradiated at room temperature and in liquid nitrogen. Additional data will be obtained with the ferrous sulfate dosimeter also irradiated at these different temperatures. A 10 cm path length sample cell has been obtained which should extend the sensitivity range of the chemical dosimeter from a lower limit of 4,000 r to 400 r.

IV. PERSONNEL

All personnel involved with this project are now back on full time employment. Approximately 2 months time was lost be R. H. Fetner as a result of the accident previously reported to your office.

V. FINANCIAL REPORT

For the period June 1 through December 31, 1961, the following expenditures were made:

Personnel Services	$5,797.72
Materials and Supplies	888.56
Travel	.00
Equipment	64.00
Freight and Express	21.30
Overhead	3,004.73
TOTAL	$9,776.31

A voucher in this amount has been submitted.

Respectfully submitted:

R. H. Fetner
Project Director

Approved:

Wyatt C. Whitley, Chief
Chemical Sciences Division

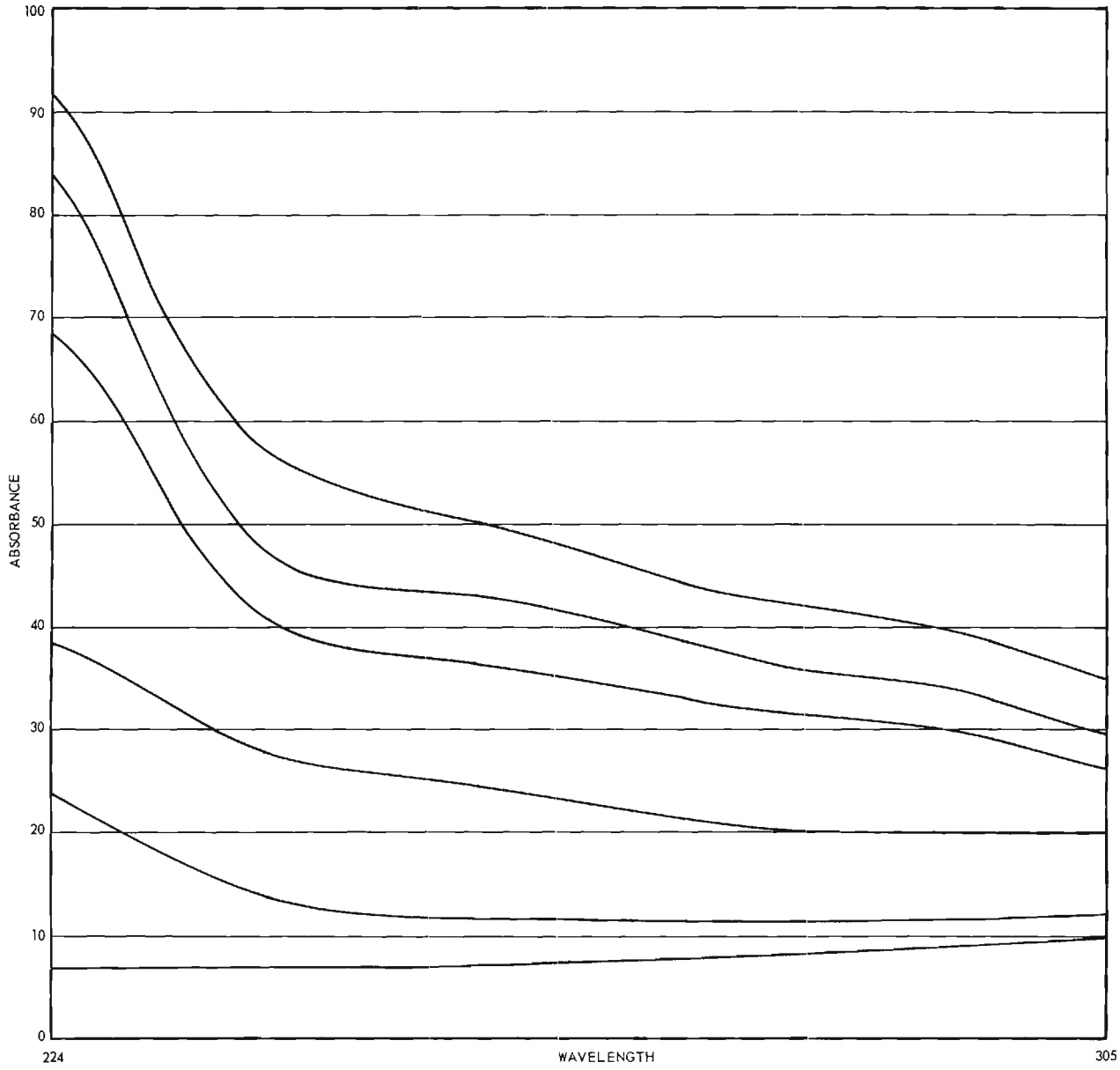


Figure 1. Typical analysis of irradiated ferrous ammonium sulfate solution at 305 mμ and 224 mμ. Beckman Model DK-2 Ratio recording spectrophometer.

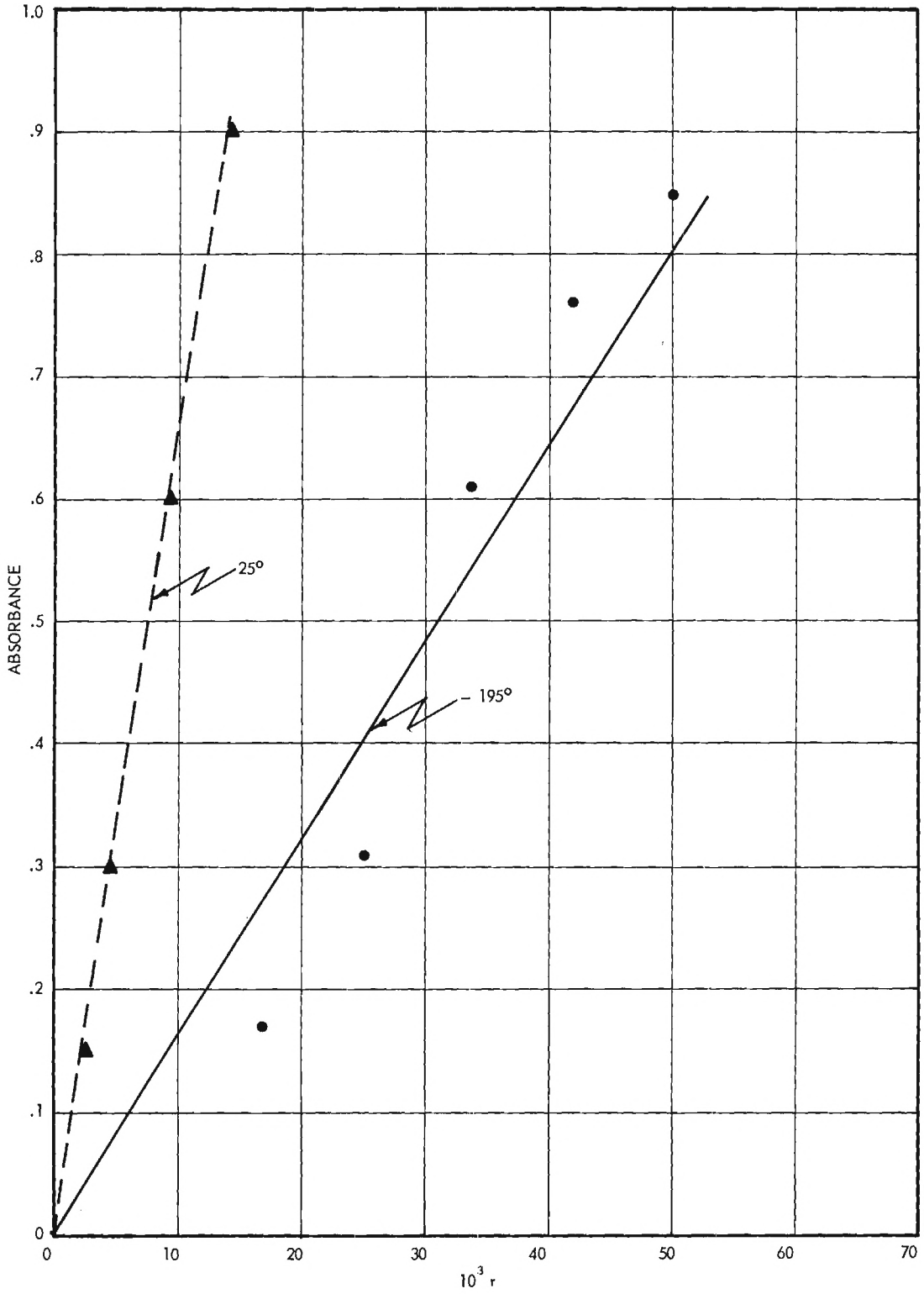


Figure 2. Optical density (224 mμ) of irradiated ferrous ammonium sulfate solution as a function of X-ray dose.

ENGINEERING EXPERIMENT STATION
of the Georgia Institute of Technology
Atlanta, Georgia

Informal Progress Report

Project A-572

RESEARCH AND REPORTS ON FROZEN HUMAN CELL CULTURES AS A TIME INDEPENDENT RADIATION DOSIMETER

By

ROBERT H. FETNER

February 1, 1962 through April 30, 1962

CONTRACT NO. AF 41(657)-408

SCHOOL OF AVIATION MEDICINE

UNITED STATES AIR FORCE
RANDOLPH AIR FORCE BASE, TEXAS
R&D PROJECT NO. 7757

I. SUMMARY

Results from analysis of slides in the 12 hr post-irradiation experiments showed the following: (1) a linear response of chromatid delections as a function of dose in the frozen and unfrozen experiments, (2) a frequency of 4.5 breaks per 10^5 chromosomes per roentgen for cells irradiated unfrozen, (3) a frequency of 1.3 breaks per 10^5 chromosomes per roentgen for cells irradiated at -190° C, (4) a mitotic inhibition induced between 200 - 400 r in the unfrozen group, (5) the lack of a mitotic inhibition with doses to 2000 r in the frozen groups.

II. FULL STATEMENT OF PROGRESS

Slide preparations made from the experimental groups of irradiated frozen and unfrozen cell cultures as described in the Formal Progress Report No. 1 are being analyzed. The slide preparations are traversed under the microscope using an oil immersion objective and phase contrast microscopy. Every metaphase chromosome viewed is included in the analysis either as a normal chromosome or as one containing a chromatid deletion. Table I presents the first data obtained on unfrozen cell cultures irradiated with 50, 100, 200 and 400 r.

Table 1

Dose (R)	No. Chromosomes (N)	No. Deletions (D)	D/N 10^{-3}	D/N/R 10^{-5}
50	4016	9	2.2	4.4
100	1827	8	4.3	4.3
200	530	5	9.4	4.7
400	-	-	-	-

This is the 12-hour post-irradiation group. At 50 r there were 9 chromatid deletions in 4,016 chromosomes, giving a frequency of 2 chromosome breaks per 1000 chromosomes. With 100 r there were 8 breaks in 1,827 chromosomes for a frequency of 4 breaks per 1000. With 200 r there were 5 breaks in 530 chromosomes for a frequency of 9 breaks per 1000. The breakage per chromosome per r was in a good agreement between these three irradiations with an average figure of above 4.5 breaks per 100,000 chromosomes per roentgen. In this group somewhere between the 200 and 400 r dose range, a mitotic inhibition was induced because in the 400 r group at the 12-hour post-irradiation interval, there were virtually no metaphase figures. Therefore, between 200 and 400 r there is a threshold dose for the induction of mitotic inhibition. In the original experimental outline it was anticipated there would be a mitotic inhibition and this was one of our primary purposes in making analyses at 12-hour post-irradiation increments.

In the frozen cell cultures irradiated at liquid nitrogen temperature the results obtained at the 12-hour post-irradiation group is presented in Table 2.

Table 2

Dose (R)	No. Chromosomes (N)	No. Deletions (D)	D/N 10^{-3}	D/N/R 10^{-5}
75	2080	2	1	1.3
300	1232	5	4.6	1.5
800	1062	11	10.4	1.3
1200	1087	17	16	1.3
2000	587	15	26	1.3

With 75 r there were 2 deletions in 2,080 chromosomes with a frequency of 1 per 1000. With 300 r there were 5 breaks in 1,232 chromosomes for a frequency of about 5 breaks per 1000. With 800 r there were 11 breaks in 1,062 chromosomes for a frequency of 10 breaks per 1000. With 1200 r there were 17 breaks in 1,087 for a frequency of 16 per 1000, and with 2000 r there were 15 breaks in 587 chromosomes for a frequency of 26 per 1000. The frequency of breaks per chromosome per r was about 1.3 per 10^5 showing a good agreement over the entire dose range.

A comparison of the breaks per chromosome per r in the frozen and unfrozen state indicates that the ratio is 4.5 for the frozen to 1.3 per 10^5 in the frozen state. This indicates a reduction of radiation sensitivity of 3.3 times in the frozen state. The production of chromatid deletions as a function of radiation dose is linear in both the frozen and unfrozen state. Of particular interest is the fact that at a dose range corresponding to the 400 r in the unfrozen state, 1200 r in the frozen state, there was no evidence of mitotic inhibition. Apparently the mitotic inhibition is not induced in cells irradiated in the frozen state, or if it is induced, it requires a higher dose than has been analyzed as yet. The analysis of the data is continuing, and the 24, 36 and 48 hour post-irradiation experimental groups are being analyzed. Also, additional quantitation is being obtained in some of the points presented in Table 1 and 2. For statistical purposes it would be desirable to obtain no less than 10 deletions per experimental group. In the case of the low irradiation levels, e.g., 75 r in the frozen state, this would require some 10,000 chromosomes to be analyzed in this group. The absence of a mitotic

inhibition in the frozen state will increase the usefulness of this system as a potential radiation dosimeter and may provide some evidence to suggest the factors responsible for the induction of mitotic inhibition by high energy radiation.

III. PLANS FOR THE FUTURE

The analysis of the slide preparations is continuing and we hope to obtain a complete analysis of all the experimental material as originally scheduled.

Respectfully submitted:

Robert H. Fetner, Ph.D.
Project Director

Approved:

Wyatt C. Whitley, Chief
Chemical Sciences Division

ENGINEERING EXPERIMENT STATION
of the Georgia Institute of Technology
Atlanta, Georgia

Informal Progress Report No. 3

Project A-572

RESEARCH AND REPORTS ON FROZEN HUMAN CELL CULTURES AS A TIME INDEPENDENT RADIATION DOSIMETER

By

ROBERT H. FETNER

May 1, 1962 through July 31, 1962

CONTRACT NO. AF 41(657)-408

SCHOOL OF AVIATION MEDICINE

UNITED STATES AIR FORCE
RANDOLPH AIR FORCE BASE, TEXAS
R&D PROJECT NO. 7757

1. SUMMARY

The determination of the frequency of chromatid deletions produced by 250 KV X-rays in frozen and unfrozen KB cells has been completed. In the unfrozen cells the optimum production of chromatid deletions was 12 hours after irradiation for doses up to 200 r. Between 200 and 400 r a mitotic inhibition was produced which resulted in the maximum number of chromatid deletions being observed 24 hours after irradiation. This was true to 1000 r, which was the maximum dose. The production of chromatid deletions as a function of x-ray dose indicated a high positive correlation between these two variables.

In the experimental group irradiated in liquid nitrogen, no mitotic inhibition was observed with doses to 2000 r. The maximum production of chromatid deletions was 12 hours after irradiation. The production of chromatid deletions as a function of dose indicated a high positive correlation between these two variables.

Cells irradiated in liquid nitrogen are 1/3 as sensitive as cells irradiated at room temperature.

2. FULL STATEMENT OF PROGRESS

Slide preparations made from the experimental groups of irradiated frozen and unfrozen cell cultures as described in the previous progress reports have now been analyzed. The slide preparations were traversed under the microscope using an oil immersion objective and phase contrast microscopy. Every metaphase chromosome viewed is included in the analysis either as a normal chromosome or as one containing a chromatid deletion. Table I presents the data obtained on unfrozen cell cultures irradiated with 50 r.

Table I

Δ Time After Irradiation (hours)	No. Chromosomes (N)	No. Deletions (D)	$\frac{D}{N} \times 10^{-3}$
12	4016	9	2.2 ± 0.7
24	2642	0	-
36	2000	0	-
48	2000	0	-
60	2000	0	-

Table I. Number of deletions after various times with 50 r - unfrozen group.

Nine deletions were observed in 4,016 chromosomes at 12 hours after irradiation. At 24, 36, 48 and 60 hours after irradiation no deletions were observed after counting 2,000 chromosomes. This indicates that there was no mitotic inhibition induced by the irradiation at this dose level or that the mitotic inhibition did not persist longer than 12 hours, otherwise, some deletions would have been evident in the 24-hour group. In Table II the results of irradiation with 100 r in the unfrozen group are presented.

Table II

Δ Time After Irradiation (hours)	No. Chromosomes (N)	No. Deletions (D)	$\frac{D}{N} \times 10^{-3}$
12	1827	8	4.4 ± 1.5
24	3400	0	-
36	2000	0	-
48	2000	0	-
60	2000	0	-

Table II. Number of deletions after various times with 100 r - unfrozen group.

Eight deletions were observed in 1,827 chromosomes 12 hours after irradiation. At 24, 36, 48 and 60 hours after irradiation there were no deletions observed with counts of at least 2,000 chromosomes. Table III gives the results of 200 r irradiation.

Table III

Δ Time After Irradiation (hours)	No. Chromosomes (N)	No. Deletions (D)	$\frac{D}{N} \times 10^{-3}$
12	991	12	12.1 ± 3.5
24	415	0	-
36	369	0	-
48	400	0	-
60	400	0	-

Table III. Frequency of deletions at various time intervals with 200 r - unfrozen group.

With 200 r in the unfrozen cell cultures, 12 deletions were observed in 991 chromosomes and, as with the 50 and 100 r experiments, no deletions were observed 24, 36, 48 and 60 hours after irradiation. Table IV summarizes the results of 400 r in the unfrozen group.

Table IV

Δ Time After Irradiation (hours)	No. Chromosomes (N)	No. Deletions (D)	$\frac{D}{N} \times 10^{-3}$
12	-	-	-
24	613	10	16.0 ± 5
36	915	6	6.5 ± 2.6
48	772	4	5.2 ± 2.5
60	-	-	-

Table IV. Number of deletions after various times with 400 r - unfrozen group.

At 12 hours after irradiation no metaphases were observed, indicating there was a mitotic inhibition which prevented cells from entering metaphase 12 hours after this dose. Twenty-four hours after irradiation there were 10 deletions observed in 613 chromosomes. At 36 hours there were 6 deletions in 915 chromosomes, and at 48 hours there were 4 deletions in 772 chromosomes. These data indicate that with 400 r irradiation in the unfrozen group a mitotic inhibition is induced which lasts longer than 12 hours and which apparently persists for at least 48 hours after irradiation in some cells. There was a peak deletion production at 24 hours after irradiation.

The effect of 600 r in the unfrozen group is presented in Table V.

Table V

Δ Time After Irradiation (hours)	No. Chromosomes (N)	No. Deletions (D)	$\frac{D}{N} \times 10^{-3}$
12	-	-	-
24	621	15	24.0 ± 6
36	666	8	12.0 ± 4.2
48	-	-	-
60	-	-	-

Table V. Number of deletions after various times with 600 r - unfrozen group.

Again there were no metaphases observed at 12 hours after irradiation. At 24 hours there were 15 deletions in 621 chromosomes. At 36 hours there were 8 deletions in 666 chromosomes.

Table VI presents the results of 800 r in the unfrozen group.

Table VI

Δ Time After Irradiation (hours)	No. Chromosomes (N)	No. Deletions (D)	$\frac{D}{N} \times 10^{-3}$
12	-	-	-
24	416	14	33.6 ± 9.0
36	670	6	8.9 ± 3.5
48	-	-	-
60	-	-	-

Table VI. Number of deletions after various times with 800 r - unfrozen group.

Deletions were observed 24 hours after irradiation where there were 14 deletions in 416 chromosomes. Thirty-six hours after irradiation there were 6 deletions observed in 670 chromosomes.

The results of 1000 r are presented in Table VII.

Table VII

Δ Time After Irradiation (hours)	No. Chromosomes (N)	No. Deletions (D)	$\frac{D}{N} \times 10^{-3}$
12	-	-	-
24	420	17	40.4 ± 10
36	800	9	11.2 ± 4
48	-	-	-
60	-	-	-

Table VII. Number of deletions after various times with 1000 r - unfrozen group.

With 1000 r in the unfrozen group, 17 deletions were observed in 420 chromosomes 24 hours after irradiation. At 36 hours 9 deletions were observed in 800 chromosomes.

The information obtained in the first seven tables is summarized in Table VIII.

Table VIII

Dose (r)	Frequency of Deletions/1000 Chromosomes after irradiation time				
	12 hr	24 hr	36 hr	48 hr	60 hr
50	2.2 ± 0.7	0	0	-	-
100	4.3 ± 1.5	0	0	-	-
200	12.0 ± 3.5	0	0	-	-
400	0	16.0 ± 5	6.5 ± 2.6	-	-
600	0	24.0 ± 6	12 ± 4.2	-	-
800	0	33.6 ± 9	8.9 ± 3.5	-	-
1000	0	40.4 ± 10	11.2 ± 4	-	-

Table VIII. Summary of data on frequency of deletions after irradiation in unfrozen group.

The threshold dose for mitotic inhibition, which persists longer than 12 hours, is between 200 and 400 r. From 400 to 1000 r the peak chromatid production occurs 24 hours after irradiation. The production of deletions drops off very sharply after 24 hours. A scatter plot of the deletion frequency as a function of irradiation dose in the unfrozen group is presented in Figure 1. The data indicate a high positive linear correlation between these two variables, and a computation was made (Table IX) to obtain an estimating equation and the standard errors (reliability) of this estimate.

Table IX

Computation of sums for correlation of roentgens of 250 KV X-rays (X) and deletions observed (Y) in chromosomes of KB cells 12 and 24 hours after irradiation

X	Y	X^2	Y^2	XY
50	2.2*	2,500	4.84	110
100	4.3*	10,000	18.49	430
200	12.0*	40,000	144.00	2,400
400	16.0**	160,000	256.00	6,400
600	24.0**	360,000	576.00	14,400
800	33.6**	640,000	1,128.96	26,880
1000	40.4**	1,000,000	1,632.16	40,400
3150	132.5	2,212,500	3,760.45	91,020

* 12 hours after irradiation

** 24 hours after irradiation

I. $\Sigma Y = Na + b\Sigma X$

II. $\Sigma XY = a\Sigma X + bX^2$

I. $132.5 = 7a + 3150b$

II. $91,020 = 3150a + 2,212,500b$

$91,020 = 3150a \quad 2,212,500b$

$59,625 = 3150a \quad 1,417,500b$

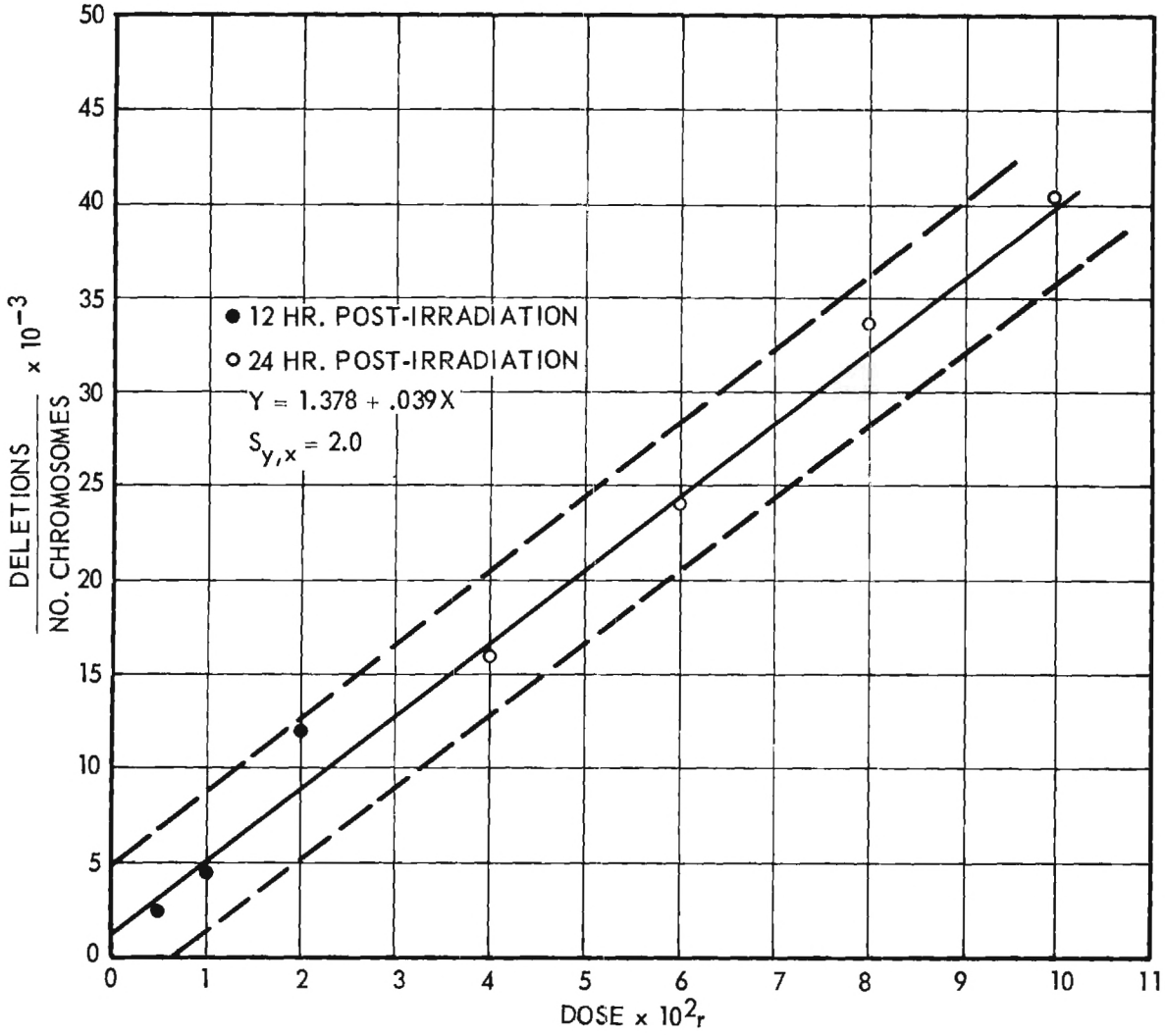
$31,405 = \quad 795,000b$

$b = .039$

$a = 1.378$

$$\boxed{Y = 1.378 + .039X}$$

Figure 1. Maximum number of chromatid deletions produced at various time increments after irradiation as a function of x-ray dose. Irradiation performed at room temperature.


50
45
40
35
30
25
20
15
10
5
0
0
1
2
3
4
5
6
7
8
9
10
11
● 12 HR. POST-IRRADIATION
○ 24 HR. POST-IRRADIATION
Y = 1.378 + .039X
$S_{y,x}$ = 2.0
DELETIONS / NO. CHROMOSOMES × 10^{-3}
DOSE × 10^2r

Irradiation in frozen group

Table X presents the data on frequency of deletions produced with 75 r in the group irradiated in liquid nitrogen.

Table X

Δ Time After Irradiation (hours)	No. Chromosomes (N)	No. Deletions (D)	$\frac{D}{N} \times 10^{-3}$
12	6131	6	0.98 ± 0.4
24	4000	0	-
36	-	-	-
48	-	-	-
60	-	-	-

Table X. Number of deletions observed after various times with 75 r - frozen group.

Twelve hours after irradiation there were 6 deletions in 6,131 chromosomes. There were no deletions observed in 4,000 chromosomes 24 hours after irradiation. The results of 300 r irradiations are presented in Table XI.

Table XI

Δ Time After Irradiation (hours)	No. Chromosomes (N)	No. Deletions (D)	$\frac{D}{N} \times 10^{-3}$
12	2464	10	4.0 ± 1.3
24	653	0	-
36	-	-	-
48	-	-	-
60	-	-	-

Table XI. Number of deletions observed after various times with 300 r - frozen group.

With 300 r there were 10 deletions produced in 2,464 chromosomes and no deletions observed in 653 chromosomes 24 hours after irradiation.

Table XII gives the results with 800 r.

Table XII

Δ Time After Irradiation (hours)	No. Chromosomes (N)	No. Deletions (D)	$\frac{D}{N} \times 10^{-3}$
12	1062	11	10.3 ± 3.2
24	523	0	-
36	-	-	-
48	-	-	-
60	-	-	-

Table XII. Number of deletions observed after various times with 800 r - frozen group.

There were 11 deletions in 1,062 chromosomes 12 hours after irradiation and, at 24 hours, there were no deletions observed in 523 chromosomes. With 1200 r, Table XIII, shows there were 17 deletions in 1,087 chromosomes 12 hours after irradiation, and 24 hours after irradiation there were no deletions observed in 620 chromosomes.

Table XIII

Δ Time After Irradiation (hours)	No. Chromosomes (N)	No. Deletions (D)	$\frac{D}{N} \times 10^{-3}$
12	1087	17	15.7 ± 3.8
24	620	0	-
36	-	-	-
48	-	-	-
60	-	-	-

Table XIII. Number of deletions observed after various times with 1200 r - frozen group.

In Table XIV the results of 2000 r show that there were 15 deletions recorded in 587 chromosomes 12 hours after irradiation. One deletion was observed in 625 chromosomes 24 hours after irradiation and no deletions were observed in 500 chromosomes at 36 hours after irradiation.

Table XIV

Δ Time After Irradiation (hours)	No. Chromosomes (N)	No. Deletions (D)	$\frac{D}{N} \times 10^{-3}$
12	587	15	25.6 ± 6.6
24	625	1	1.6
36	500	0	-
48	-	-	-
60	-	-	-

Table XIV. Number of deletions observed various times after 2000 r - frozen group.

The data on irradiation in the frozen group is summarized in Table XV. With irradiation up to 2000 r there was no mitotic inhibition detected as was evident between 200 and 400 r in the unfrozen group.

Table XV

Dose (r)	Frequency of Deletions/1000 Chromosomes after irradiation time				
	12 hr	24 hr	36 hr	48 hr	60 hr
75	0.98 ± 0.4	0	-	-	-
300	4.0 ± 1.3	0	-	-	-
800	10.3 ± 3.2	0	-	-	-
1200	15.7 ± 3.8	0	-	-	-
2000	25.6 ± 6.6	1.6	0	-	-

Table XV. Summary of data on frequency deletions noticed after irradiation in frozen group.

Figure 2 shows a scatter plot of the frequency of chromatid deletions as a function of x-ray dose in the cultures irradiated in liquid nitrogen. An equation was estimated to correlate these two data variables by the method of the least squares and the data used in this computation are presented in Table XVI. These data indicate a very good correlation between these two variables.

Table XVI

Computation of sums for correlation of 250 KV X-ray dose (X) and deletions observed (Y) in chromosomes of KB cells 12 hours after irradiation

X	Y	X^2	Y^2	XY
75	0.98	5,625	0.96	73.4
300	4.0	90,000	16.00	1,200.0
800	10.3	640,000	106.09	8,240.0
1200	15.7	1,440,000	246.49	18,840.0
2000	25.6	4,000,000	655.36	51,200.0
4375	56.58	6,175,625	1,024.90	79,553.4

I. $\Sigma Y = Na + b\Sigma X$

II. $\Sigma XY = z\Sigma X + bX^2$

I. $56.58 = 5a + 4375b$

II. $79553.4 = 4375a + 6,175,625b$

$79553.4 = 4375a + 6,175,625b$

$49507.5 = 4375a + 3,828,125b$

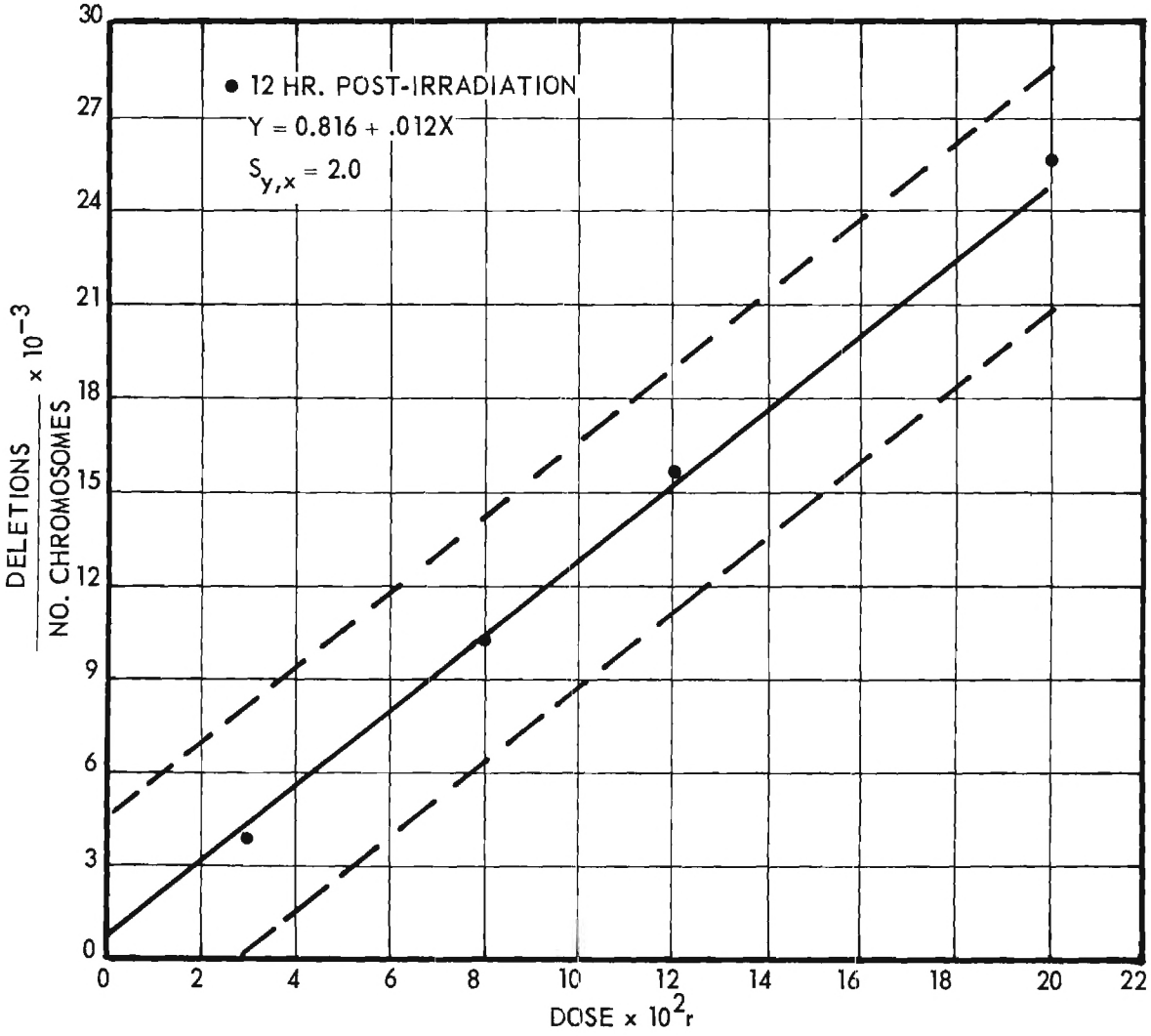
$30045.9 = 2,347,500b$

$b = .012$

$a = .816$

$$\boxed{Y = .816 + .012X}$$

Figure 2. Maximum number of chromatid deletions produced at various time increments after irradiation as a function of x-ray dose. Irradiation performed with cells in liquid nitrogen (-190°C).


● 12 HR. POST-IRRADIATION
Y = 0.816 + .012X
$S_{y,x}$ = 2.0
DELETIONS / NO. CHROMOSOMES × 10^{-3}
DOSE × 10^2r
0
3
6
9
12
15
18
21
24
27
30
0
2
4
6
8
10
12
14
16
18
20
22

3. PLANS FOR THE FUTURE

Experiments have been completed and slide preparations made in the fractionation experiments. 1000 r in non-frozen cultures was delivered in two equal doses of 500 r each with 1-, 2-, 4- and 8-day increments between the two doses. Slide preparations were made 12 and 24 hours after the last irradiation. In the frozen group two equal doses of 500 r were also delivered with 1-, 2-, 4- and 8-day increments between the two doses, and slide preparations made as in the unfrozen group. These slides are now being analyzed to determine if the cells in the frozen condition will be time-independent as determined by dose fractionation.

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Informal Progress Report No. 4

Project No. A-572

RESEARCH AND REPORTS ON FROZEN HUMAN CELL CULTURES AS A TIME INDEPENDENT RADIATION DOSIMETER

By

ROBERT H. FETNER

August 1, 1962 through October 31, 1962

CONTRACT NO. AF 41(657)-408

SCHOOL OF AVIATION MEDICINE

UNITED STATES AIR FORCE
RANDOLPH AIR FORCE BASE, TEXAS
R&D PROJECT NO. 7757

1. SUMMARY

Additional work has been performed with the Fricke dosimeter. A 10 cm path length cell has been incorporated and extends the usable range of the dosimeter down into the range of greatest biological interest. With this technique there was a high positive correlation between dose and absorbance. At the point of greatest sensitivity of the spectrophotometer, 0.3 absorbance, the yield of Fe^{+++} ions indicates a dose of 500 ± 10 r.

Irradiation of the dosimeter at -190°C resulted in a reduction of Fe^{+++} ion yield. The results were not reproducible from experiment to experiment or between replicate samples. The addition of glycerol did not markedly increase the yield of Fe^{+++} ions in the frozen state and did not increase the reproductibility.

2. FULL STATEMENT OF PROGRESS

Experimental work has been continuing on the irradiation of the Fricke chemical dosimeter as a comparison for the frozen cell culture dosimeter used in the study. It was hoped that information obtained with a known chemical system under conditions comparable with the biological experimentation might give some insight as to the nature of the responses obtained in the biological system. This is a continuation of the work which had been reported in Formal Progress Report No. 1. The following have been performed:

(1) The utilization of a 10 centimeter path length sample cell in the spectrophotometer to extend the range of the chemical dosimeter from a lower limit of 4000 r to about 400 r. A series of experiments were run determining how reproducible the results are using this sample size at the lower dose ranges.

(2) Additional experimentation comparing the ferric ion yields in the Fricke dosimeter at room temperature and -190°C.

(3) Investigation of the effect of the addition of glycerol to the Fricke dosimeter and its effect on the yield of ferric ions in the frozen state.

(1) Experiments with the Fricke dosimeter using a 10 centimeter path length absorption cell.

In these experiments a ferrous ammonium sulfate solution was made up according to the following recipe:

2 grams of ferrous ammonium sulfate
.3 grams of sodium chloride
110 milliliters of concentrated reagent grade sulfuric acid in sufficient distilled water to make 5 liters of solution

This same recipe was followed in making up all of the dosimeter solutions. The solutions were irradiated in open-top DeWar flasks and the dose was determined using a Victoreen R meter. After radiation the absorbance was determined at 224 mμ as has been suggested by Sharpe and Lee[1] who claim a two-fold increase in sensitivity at this wavelength due to the high moler extension coefficient which is 4565 liters per mole centimeter at 224 mμ as compared to 2196 liters per mole centimeter at 304 mμ at 25°C. An additional advantage of the 224 mμ analysis is the much lower temperature dependence which is 0.1 per cent at 224 mμ as compared to .7 per cent per degree centigrade at 304 mμ. In Table I the results of irradiation experiments performed on several different days are presented. There is a high positive correlation between dose and absorbance, indicating a good reproducibility of this experimental procedure and also indicating that increasing the path length to 10 centimeters extends the useful range of this chemical dosimeter down into the range of greatest biological interest. At the point of greatest sensitivity of the spectrophotometer,

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1 Scharf, K. and R. M. Lee. Rad. Research 14: 498 (1961).

Table I

Absorbance (Optical density)	Dose (r)	Theoretical dose*
.08	125	117
.11	130	162
.16	250	232
.24	375	353
.23	400	338
.33	500	500
.33	500	500
.42	625	617
.44	650	647
.50	750	735
.53	780	780
.60	875	880
.60	910	880
.70	1000	1030
.67	1000	985
.68	1040	1000
.82	1170	1200

*Absorbance x 1470 = dose

Least squares analysis: absorbance = 1.70 + .069 dose

$S_{dose,\ absorbance} = 10$

Table I. Absorbance of Fe^{+++} ions as a function of radiation dose in a 10 cm absorption cell.

0.3 optical density (50% transmission), the dose = 500 ± 10 r, indicating a 2% variance.

(2) Experiments with frozen dosimeter.

In these experiments 10 ml ampules were filled with ferrous ammonium sulfate solution and heat-sealed. The ampules were frozen and irradiated submerged in liquid nitrogen in open-top DeWar flasks. After irradiation the ampules were thawed and the concentration of ferric ion measured in the spectrophotometer. The results of several of these experiments is presented in Table II.

Table II

Absorbance (Optical Density)	Dose (r)	Theoretical dose*
.04	100	59
.09	240	132
.09	400	132
.18	520	262
.12	520	176
.20	650	294
.26	780	382
.20	780	294
.30	920	440
.32	1020	470
.40	1180	587

*Absorbance x 1470 = dose in r

Table II. Absorbance of Fe^{+++} ions as a function of radiation dose in a 10 cm absorption cell. Solutions irradiated at -190°C.

In all cases the yield of ferric ions as determined by absorbance at 224 mμ was less when irradiation was performed at liquid nitrogen temperatures than at room temperature. The yield of ferric ions as a function of radiation dose in these experiments, however, was not reproducible from experiment to experiment, and even within an experimental group. The yield did not appear to be linear with dose. It may be that a number of factors could influence the yield of ferric ions, including rate of freezing and rate of thawing. It was brought to our attention also that there is a tendency to freeze out dissolved materials during the formation of ice crystals. In this case there would be an actual freezing out of the ferrous ions and an unequal concentration of them in the solution.

(3) The addition of glycerol to the ferrous ammonium sulfate solution before freezing.

The effects of the addition of glycerol was examined in a preliminary investigation. Insofar as glycerine is used in freezing of the biological dosimeter, a preliminary investigation seemed worthwhile. It is known that the addition of organic materials to the Fricke dosimeter results in an increased yield with considerable evidence to suggest that as many as 3 ferric ions can be reoxidized as the result of an organic intermediate radical[2] and the justification for the addition of sodium chloride to the ferrous ammonium sulfate solution is the result of a demonstration that it suppresses the effect of organic contaminants through a differential oxidation of the chloride[2]. We, however, wished to investigate the effect of the glycerine primarily in the frozen state because of its known effect on ice crystal formation.

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2 Bacq, Z. M. and P. Alexander. Fundamentals of Radiobiology. Pergamon Press, New York (1961).

The preliminary results of these experiments are presented in Table II. The presence of the glycerine precluded the use of the 10 centimeter cells, there being insufficient energy in our spectrophotometer to traverse the glycerine solution. Experiments were performed at higher dose rates using a 1 centimeter path length cell. The results are presented in Table III and it can be seen that there was no improvement in reproducibility either between replicate samples or between experimental points determined on separate days. It would appear that the addition of glycerine did not markedly increase yield in the frozen state and it did not increase the reproducibility.

It would appear that without additional work involving extensive experimentation on the rates of freezing, rates of thawing and the effect of other factors on the frozen dosimeter that the system is not reproducible enough to yield useful information pertinent to an understanding of events transpiring in the biological dosimeter.

Table III

Absorbance (Optical Density)	Dose (r)	Theoretical dose*
.58	5000	8232
.64	5000	9408
.70	7500	1029
.64	7500	9408
.91	10,000	13,230
.82	10,000	12,056
.19	5000	2800
.13	5000	1900
.26	7500	3820
.22	7500	3230
.33	10,000	5000
.26	10,000	3800

*Absorbance x 14700 = dose in r

Table III. Absorbance of Fe^{+++} ions as a function of radiation dose in a 1 cm absorption cell. Solutions contained 10% (volume/volume) glycerol.

3. PLANS FOR THE FUTURE

Work is continuing on the effects of irradiation on frozen cell cultures. A series of dose fractionation experiments has been completed and the slides are being analyzed. Two doses of 500 r were separated by increments of 0.5, 1, 2, 4, 8 and 30 days and cultures made after the second irradiation. To test the effect of post-irradiation storage, a group of frozen cell cultures were irradiated with 1000 r and are being tested at 30-day intervals.

A paper based on our work completed thus far on this project has been accepted for presentation for the 1963 meeting of the Aerospace Medical Association. The title of the paper will be "The effect of cold on the time and frequency of radiation-induced chromosome deletions."

Another paper on the same subject has been completed and will be submitted to the School of Aviation Medicine for their consideration.

Respectfully submitted:

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Approved:

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ENGINEERING EXPERIMENT STATION
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Informal Progress Report No. 5

Project No. A-572

RESEARCH AND REPORTS ON FROZEN HUMAN CELL CULTURES AS A TIME INDEPENDENT RADIATION DOSIMETER

By

ROBERT H. FETNER

November 1, 1962 through January 31, 1963

CONTRACT NO. AF 41(657)-408

SCHOOL OF AVIATION MEDICINE

UNITED STATES AIR FORCE
RANDOLPH AIR FORCE BASE, TEXAS
R&D PROJECT NO. 7757

1. SUMMARY

Dose fractionation experiments have been completed using two doses of 500 r separated with increments of 0.5, 1, 2, 4, 8 and 30 days. The yield of interstitial deletions per chromosome was not significantly different from that of a single 1000 r dose.

2. FULL STATEMENT OF PROGRESS

The slide preparations of the dose fractionation experiments have been analyzed and the data are presented in Table I. In these experiments ampules of cells were irradiated with 500 r of 250 KV X-rays at -190° and then after increments of 0.5, 1, 2, 4, 8 and 30 days a second dose of 500 r was administered to the cultures. They were then rapidly thawed and placed in culture bottles at 35°C. At 12 hrs the cultures were agitated to harvest the metaphase cells, hypotonically treated, fixed, and stained, and slide preparations were made. The frequency of interstitial deletions was determined in each group and there was not a significant difference between the dose fractionation yield of frequency of deletions and the previously determined figure for aberrations per 1000 r which was equal to 12.8×10^{-3} deletions per chromosome. Over this period of time there is apparently no effect of dose fractionation on yield of interstitial chromosome deletions.

A paper entitled "Chromatid Deletions Produced in Human Cell Cultures by 250 KV X-rays at Liquid Nitrogen Temperatures," was completed and was submitted to the Aerospace Medical Association. A copy of this paper is attached in the supplement to this report.

Table I

Time between dose fractions (days)	Number of Chromosomes	Frequency of Interstitial Chromatid Deletions x 10^{-3}	"t" Table level of significance
0*	-	12.8 ± 0.8	-
0.5	1019	12.2	.60
1.0	818	13.4	.60
2.0	971	13.7	.50
4.0	1011	12.1	.50
8.0	853	13.9	.40
30.0	975	12.4	.60

*Single dose of 1000 r (see Progress Report No. 3) frequency determined from estimating equation; freq. = .816 ± .012 dose.

Table I. Frequency of interstitial chromatid deletions produced by two doses of 500 r of 250 KV X-rays separated by various increments of time.

3. PLANS FOR THE FUTURE

The dose intensity experiments are being performed and the analysis of the data is under way.

It would be highly desirable to duplicate the work performed on this project with fast neutrons which will soon be available at our new Nuclear Reactor complex.

Respectfully submitted:

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Approved:

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SUPPLEMENT

CHROMATID DELETIONS PRODUCED IN HUMAN CELL CULTURES BY 250 KV X-RAYS AT LIQUID NITROGEN TEMPERATURES

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ABSTRACT

The frequency of interstitial chromatid deletions produced by 250 KV X-rays was studied in KB cell cultures at -190°C and 25°C. In unfrozen cells the optimum production of chromatid deletions was 12 hours after irradiation for doses up to 200 r. Between 200 and 400 r a mitotic inhibition was produced which resulted in the maximum number of chromatid deletions being observed 24 hours after irradiation. This was true to 1000 r, which was the maximum dose. The production of chromatid deletions as a function of X-ray dose indicated a high positive correlation between these two variables. There were 4×10^{-5} interstitial deletions/chromosome/r in the unfrozen group.

In the experimental group irradiated in liquid nitrogen, no mitotic inhibition was observed with doses to 2000 r. The maximum production of chromatid deletions was 12 hours after irradiation. The production of chromatid deletions as a function of dose indicated a high positive correlation between these two variables. There were 1.3×10^{-5} interstitial deletions/chromosome/r in the frozen group.

Cells irradiated in liquid nitrogen are 1/3 as sensitive to chromosome breakage as cells irradiated at room temperature.

CHROMATID DELETIONS PRODUCED IN HUMAN CELL CULTURES BY 250 KV X-RAYS AT LIQUID NITROGEN TEMPERATURES

In studies of radiation-induced chromosome aberrations it is desirable to analyze for damage in the first metaphase after irradiation. Analyses carried beyond the first division after irradiation make quantitation difficult because of the rapidly decreasing frequency of aberrations at subsequent metaphases (13,21). Mitotic inhibition may also be superimposed over the effect desired. This requirement, therefore, imposes a considerable time limitation on experimental procedures, particularly in rapidly dividing cell populations. The investigations reported here have as one of their primary objectives to determine if frozen cells would permit an independence of this time limitation imposed in rapidly proliferating cell lines. Recent advances in techniques for freezing cell cultures now permit a high frequency of cell recovery from the frozen state even after several years' storage (20). Specific references to the production of chromosome aberrations after irradiation at low temperatures are limited to several brief reports of Faberge (9, 10,11).

METHOD

The KB human cell line (6) used in these investigations was grown in a medium composed of Eagle's amino acid and vitamin solution (7) in Hank's salt solution to which 10 per cent pooled human serum had been added. All cultures were incubated at 35°C. Cell cultures to be frozen were in the logarithmic growth phase and were harvested by scraping from glass prescription bottles and suspended in a medium composed of 50 per cent Hank's saline, 40 per cent

pooled human serum, and 10 per cent glycerol. Cells from an entire culture bottle were heat sealed in a 5 ml glass ampule, frozen at a constant rate of -1° per minute until they reached -30°C, transferred to liquid nitrogen and placed in a liquid nitrogen refrigerator (-190°C). Cells frozen in this manner show better than 90 per cent recovery upon thawing and plating on glass surfaces. More important to this investigation, such cells readily adhere to the glass surface after thawing and apparently are as physiologically active as fresh transfers which have not been frozen. Irradiations were performed with 250 KV, 15 ma X-rays in a constant potential X-ray unit (Westinghouse). All irradiations used 1 mm of aluminum and 1 mm of copper filtration with a half layer value of 2 mm of aluminum. Dose was determined with a Victoreen "R" meter and the dose rate was approximately 80 r per minute in the various experiments. Frozen cultures were irradiated in liquid nitrogen while in an open-top DeWar flask. After irradiation ampules were removed from liquid nitrogen and within several minutes immersed in 35°C water for rapid thawing. Cells were removed from the ampules, centrifuged at 500 rpm for 5 minutes, the saline-serum-glycerol medium decanted, re-suspended in growth medium and incubated at 35°C in prescription bottles. Subsequently, the cells in metaphase were harvested every 12 hours by agitating the culture and decanting the medium from the prescription bottles on which the cells were growing attached to the surface. It has been shown that this technique will harvest the metaphase cells which, because of their rounded condition, are easily dislodged from the glass surface (2). Thus, it is possible to harvest cells in metaphase without sacrificing the entire culture. Medium containing the metaphase cells was then diluted to 20 per cent concentration with water, kept at a

constant temperature (35°C) for 20 minutes and then added to at least 3 volumes of a propionic acid--ethanol (1:3) solution. The cells were then concentrated by centrifugation in a conical bottom centrifuge tube and re-suspended in a solution of propionic acid--orcein. This staining solution was freshly prepared by saturating a 50 per cent aqueous solution of propionic acid with orcein, boiling and filtering after the solution had cooled. Cells were then re-centrifuged, the staining solution decanted and the cell pellet transferred to a slide and pressed with a cover slip until well-flattened metaphase figures suitable for detailed chromosome analysis were obtained (Figure 1). The preparations were then made permanent with the dry ice technique. Our particular slide preparation technique was selected after preliminary studies (12) had indicated the desirability of using interstitial chromatid deletions as the criteria for radiation effect. The use of interstitial deletions has been criticized as representing "achromatic regions" or "synthesis gaps" (4,16,18) and were excluded by these workers in scoring chromatid aberrations. The true nature of this effect remains to be determined. We have found it to be the easiest chromosome damage to identify in this cell type and place a corresponding confidence in its use as a radiation dosimeter.

In our analysis we made no attempt to record the particular cell from which the chromosomes came, but treated the chromosomes as a population. Metaphase cells were harvested and slide preparations made in this manner at 12 hour intervals from each culture bottle for 36 hours after irradiation. In this way it was possible to determine if there was a peak production of deletions after irradiation and how long this peak persists. This permitted a maximum figure to be obtained for aberrations as a function of dose and

prevented errors from being introduced as a result of such physiological processes as mitotic inhibition which were more prevalent at some doses than others. The experiments of irradiation in unfrozen cultures were treated in exactly the same way as the frozen cell cultures except that the ampules were not frozen.

RESULTS

The results of irradiation experiments performed at room temperature (25°C) with various doses are summarized in Table I. With doses of 50, 100 and 200 r the maximum chromatid deletion production was obtained 12 hours after irradiation. Twenty-four hours after irradiation with the 50, 100 and 200 r group no chromatid deletions were observed; however, chromosome-type aberrations were seen in this group including dicentric and ring chromosomes and their acentric fragments (Figure 1). With doses of 400, 600, 800 and 1000 r no metaphases were observed with the 12 hour harvest of cells, but chromatid deletions were detected in the 24 hour group. Thirty-six hours after irradiation chromatid deletions were still visible as were chromosome-type aberrations, both occurring in the same population of chromosomes.

Table II presents the data from irradiation experiment on frozen cells. Cells were analyzed at 12, 24 and 36 hours after receiving doses of 75, 300, 600, 1200 and 2000 r while immersed in liquid nitrogen. In this entire group the maximum number of chromatid deletions were found to occur 12 hours after irradiation. No interstitial chromatid deletions were observed 24 hours after irradiation except in the 2000 r group.

Figure 2 is a diagram of the frequency of chromatid deletions produced as a function of dose in the radiation experiments. The points used represent the maximum production of chromatid deletions detected after irradiation. In the unfrozen group for 50, 100 and 200 r this was 12 hours after irradiation and for 400, 600, 800 and 1000 r this was 24 hours after irradiation. In the frozen group the maximum chromatid production was 12 hours after irradiation of all doses. The estimating equation determined by the method of the least squares was $Y = 1.378 + .039X$ for the unfrozen cells and $Y = .816 + .012\ X$ for the frozen group; this is indicated by the solid lines and twice the standard error of the fitted lines is represented by the broken lines. There was a high positive linear correlation between interstitial chromatid deletions and radiation dose in both experimental groups.

Under the conditions of these experiments a mitotic inhibition was produced in the unfrozen cell cultures between doses of 200 - 400 r which persisted for longer than 12 hours and resulted in the maximum production of chromatid deletions to be produced 24 hours after irradiation with doses of 400 to 1000 r. Cell irradiation in liquid nitrogen with doses to 2000 r did not exhibit a mitotic inhibition which lasted as long as 12 hours.

There were 4×10^{-5} interstitial deletions/chromosome/r in the unfrozen group and 1.3×10^{-5} interstitial deletions/chromosome/r in the frozen group. Cells irradiated in liquid nitrogen are about 1/3 as sensitive as unfrozen cells as determined by the production of interstitial chromatid deletions.

TABLE I. Frequency of interstitial deletions at various times after irradiation in unfrozen group. Standard errors were determined by S.E. $= \sqrt{\frac{A}{N}}$ where A = freq. aberrations and N = number of chromosomes.

	Time After Irradiation					
	12 hr		24 hr		36 hr	
Dose (r)	No. Chrom.	Frequency deletions $X10^{-3}$	No. Chrom.	Frequency deletions $X10^{-3}$	No. Chrom.	Frequency deletions $X10^{-3}$
50	4016	2.2 ± 0.7	2642	0	2000	0
100	1827	4.4 ± 1.5	3400	0	2000	0
200	991	12.1 ± 3.5	415	0	369	0
400	*	*	613	16.0 ± 5.0	915	6.5 ± 2.6
600	*	*	621	24.0 ± 6.0	666	12.0 ± 4.2
800	*	*	416	33.6 ± 9.0	670	8.9 ± 3.5
1000	*	*	420	40.4 ± 10.0	800	11.2 ± 4.0

*No metaphase figures observed.

TABLE II. Frequency of interstitial deletions at various times after irradiation in liquid nitrogen. The 36-hour group were not analyzed when there were no deletions observed in the 24-hour group.

	Time After Irradiation					
	12 hr		24 hr		36 hr	
Dose (r)	No. Chrom.	Frequency deletions $X10^{-3}$	No. Chrom.	Frequency deletions $X10^{-3}$	No. Chrom.	Frequency deletions $X10^{-3}$
75	6131	0.9 ± 0.4	4000	0	-	-
300	2464	4.0 ± 1.3	653	0	-	-
800	1062	10.3 ± 3.2	523	0	-	-
1200	1087	15.7 ± 3.8	620	0	-	-
2000	587	25.6 ± 6.6	625	1.6	500	0

DISCUSSION

The reduction in yield of chromosome breakage as a function of radiation dose in frozen cells reported in these data is different from that reported by Faberge. Our data indicates a three-fold decrease in sensitivity in the frozen state as compared with a five-fold (1950) and two-fold (1954) decrease from the reports of Faberge (10,11), although different biological systems are involved -- the work of Faberge having been performed with Tradescantia pollen. These reports appear to be the only ones available on the production of chromosome breakage at very low temperatures. Other biological and chemical systems have been studied, however, and the work with bacteria, for example (14, 19), indicates that the irradiation sensitivity decreases to about 1/3 between room temperature and liquid air temperature for E. coli. This same magnitude of sensitivity reduction appears to be characteristic for the inactivation of dry bacteriophage (3), dry catalase (17), the breakage of carbon to carbon bonds in polyisobutylenes (1), and irradiation of dry spores of Bacillus megaterium (22).

The demonstration that a mitotic inhibition was induced which lasted for longer than 12 hours after irradiation with 200 to 400 r in the unfrozen cell cultures is in general agreement with other reports (5,8,15). Radiation-induced mitotic inhibition is known to have a threshold value and the duration of the inhibition has been demonstrated to be dose dependent. The failure of cells in the frozen state to demonstrate a mitotic inhibition even at very high doses is significant. The exact nature of the mitotic inhibition is unknown but the presence of a threshold value (multihit effect) has been interpreted as evidence that it may be the result of an interference with

some metabolic process. The protection which the frozen state provides, as evidenced in these data, may indicate that the mitotic inhibition is a result of events which take place in that part of the cell rendered inactive (water?) by very low temperatures.

Frozen human cells may provide a useful time-independent dosimeter which can accumulate radiation over long periods of time and then permit a cytological analysis at the next metaphase. A final evaluation will await determination of dose intensity and fractionation studies as well as the effects of post-irradiation storage. These factors are under investigation and will be reported later.

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FINAL REPORT

PROJECT NO. A-572

RESEARCH AND REPORTS ON FROZEN HUMAN CELL CULTURES AS A TIME INDEPENDENT RADIATION DOSIMETER

By Robert H. Fetner

Prepared for
School of Aviation Medicine
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Engineering Experiment Station
GEORGIA INSTITUTE OF TECHNOLOGY
Atlanta, Georgia

ENGINEERING EXPERIMENT STATION
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Atlanta, Georgia

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SCHOOL OF AVIATION MEDICINE

UNITED STATES AIR FORCE
RANDOLPH AIR FORCE BASE, TEXAS
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JUNE 30, 1963

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I. ABSTRACT

Cultures of the KB human cell line were irradiated with 250 KV X-ray at room temperature and in liquid nitrogen. After irradiation the cells were plated in glass bottles and metaphase cells harvested, by agitation, at 12 hr intervals. The following results were obtained:

(1) a linear response of interstitial chromatid deletions as a function of dose in the frozen and unfrozen experiments,

(2) a frequency of 4.5 breaks per 10^5 chromosomes per roentgen for cells irradiated unfrozen,

(3) a frequency of 1.3 breaks per 10^5 chromosomes per roentgen for cells irradiated at -190°C,

(4) a mitotic inhibition induced between 200 - 400 r in the unfrozen group,

(5) the lack of a mitotic inhibition with doses to 2000 r in the frozen groups.

The effects of dose fractionation on frozen cells was investigated by separating two doses of 500 r with increments of 0.5, 1, 2, 4, 8 and 30 days. The yield of interstitial chromatid deletions was not significantly different from that of a single 1000 r dose. The effects of dose intensity on frozen cells was also investigated by varying the dose intensity of a total 1000 r dose by 100, 10 and 1 r per minute. The yield of interstitial chromatid deletions did not differ significantly between the various intensity experiments.

Radiation experiments were also performed using the Fricke dosimeter at room temperature and -190°C.

Irradiation of the dosimeter at -190°C resulted in a reduction of Fe^{+++} ion yield. However, the results were not reproducible from experiment to experiment or between replicate samples. It was found that by using a 10 cm path length cell and a wave length of 224 mμ on the spectrophotometer the usable range of the dosimeter could be extended down into the range of greatest biological interest. With this technique there was a high positive correlation between dose and absorbance. At the point of greatest sensitivity of the spectrophotometer, 0.3 absorbance, the yield of Fe^{+++} ions indicated a dose of 500 ± 10 r.

II. MATERIALS AND METHODS

A. MAINTENANCE AND FREEZING OF CELL CULTURES.

The KB human cell line used in these investigations was grown in a medium composed of Eagle's amino acid and vitamin solution in Hank's salt solution to which 10 per cent pooled human serum had been added. Cultures were incubated at 35°C. Cell cultures to be frozen were in the logarithmic growth phase and were harvested by scraping from glass prescription bottles and suspended in a medium composed of 50 per cent Hank's saline, 40 per cent pooled human serum, and 10 per cent glycerol. Cells from an entire culture bottle were heat sealed in a 5 ml glass ampule, frozen at a constant rate of -1° per minute until they reached -30°C, transferred to liquid nitrogen and placed in a liquid nitrogen refrigerator (-190°C). Cells frozen in this manner show better than 90 per cent recovery upon thawing and plating on glass surfaces. More important to this investigation, such cells readily adhere to the glass surface after thawing and apparently are as physiologically active as fresh transfers which have not been frozen.

B. IRRADIATION PROCEDURES.

Irradiations were performed with 250 KV, 15 ma X-rays in a constant potential X-ray unit (Westinghouse). All irradiations used 1 mm of aluminum and 1 mm of copper filtration with a half layer value of 2 mm of aluminum. Dose was determined with a Victoreen "R" meter and the dose rate was approximately 80 r per minute in the various experiments. Frozen cultures were irradiated in liquid nitrogen while in an open-top DeWar flask. After irradiation ampules were removed from liquid nitrogen and within several minutes immersed in 35°C water for rapid thawing. Cells were removed from the

ampules, centrifuged at 500 rpm for 5 minutes, the saline-serum-glycerol medium decanted, re-suspended in growth medium and incubated at 35°C in prescription bottles. Subsequently, the cells in metaphase were harvested every 12 hours by agitating the culture and decanting the medium from the prescription bottles on which the cells were growing attached to the surface. It has been shown that this technique will harvest the metaphase cells which, because of their rounded condition, are easily dislodged from the glass surface. Thus, it is possible to harvest cells in metaphase without sacrificing the entire culture.

C. SLIDE PREPARATION AND ANALYSIS.

Medium containing the metaphase cells was diluted to 20 per cent concentration with water, kept at a constant temperature (35°C) for 20 minutes and then added to at least 3 volumes of a propionic acid-ethanol (1:3) solution. Cells were then concentrated by centrifugation in a conical bottom centrifuge tube and re-suspended in a solution of propionic acid-orcein. This staining solution was freshly prepared by saturating a 50 per cent aqueous solution of propionic acid with orcein, boiling and filtering after the solution had cooled. Cells were then re-centrifuged, the staining solution decanted and the cell pellet transferred to a slide and pressed with a cover slip until well-flattened metaphase figures suitable for detailed chromosome analysis were obtained. The preparations were then made permanent with the dry ice technique. Our particular slide preparation technique was selected after preliminary studies had indicated the desirability of using interstitial chromatid deletions as the criteria for radiation effects. The use of interstitial deletions has been criticized as representing

"achromatic regions" or "synthesis gaps" and were excluded by these workers in scoring chromatid aberrations. The true nature of this effect remains to be determined. We have found it to be the easiest chromosome damage to identify in this cell type and place a corresponding confidence in its use as a radiation dosimeter.

In our analysis we made no attempt to record the particular cell from which the chromosomes came, but treated the chromosomes as a population. Metaphase cells were harvested and slide preparations made in this manner at 12-hour intervals from each culture bottle for 36 hours after irradiation. In this way it was possible to determine if there was a peak production of deletions after irradiation and how long this peak persists. This permitted a maximum figure to be obtained for aberrations as a function of dose and prevented errors from being introduced as a result of such physiological processes as mitotic inhibition which were more prevalent at some doses than others. The experiments of irradiation in unfrozen cultures were treated in exactly the same way as the frozen cell cultures except that the ampules were not frozen.

D. PREPARATION OF CHEMICAL DOSIMETER AND IRRADIATION PROCEDURE.

The ferrous ammonium sulfate solution was made up according to the following recipe:

2 grams of ferrous ammonium sulfate
.3 grams of sodium chloride
110 milliliters of concentrated reagent grade sulfuric acid in sufficient distilled water to make 5 liters of solution

This same recipe was followed in making up all of the dosimeter solutions. The solutions were irradiated in open-top DeWar flasks and the dose was determined using a Victoreen R meter.

E. ANALYTICAL TECHNIQUES.

After radiation the absorbance was determined at 224 mμ as has been suggested by Sharpe and Lee who claim a two-fold increase in sensitivity at this wavelength due to the high moler extension coefficient which is 4565 liters per mole centimeter at 224 mμ as compared to 2196 liters per mole centimeter at 304 mμ at 25°C. An additional advantage of the 224 mμ analysis is the much lower temperature dependence which is 0.1 per cent at 224 mμ as compared to .7 per cent per degree centigrade at 304 mμ. Figure 1 is a typical analysis of several radiation experiments at the two different wave lengths.

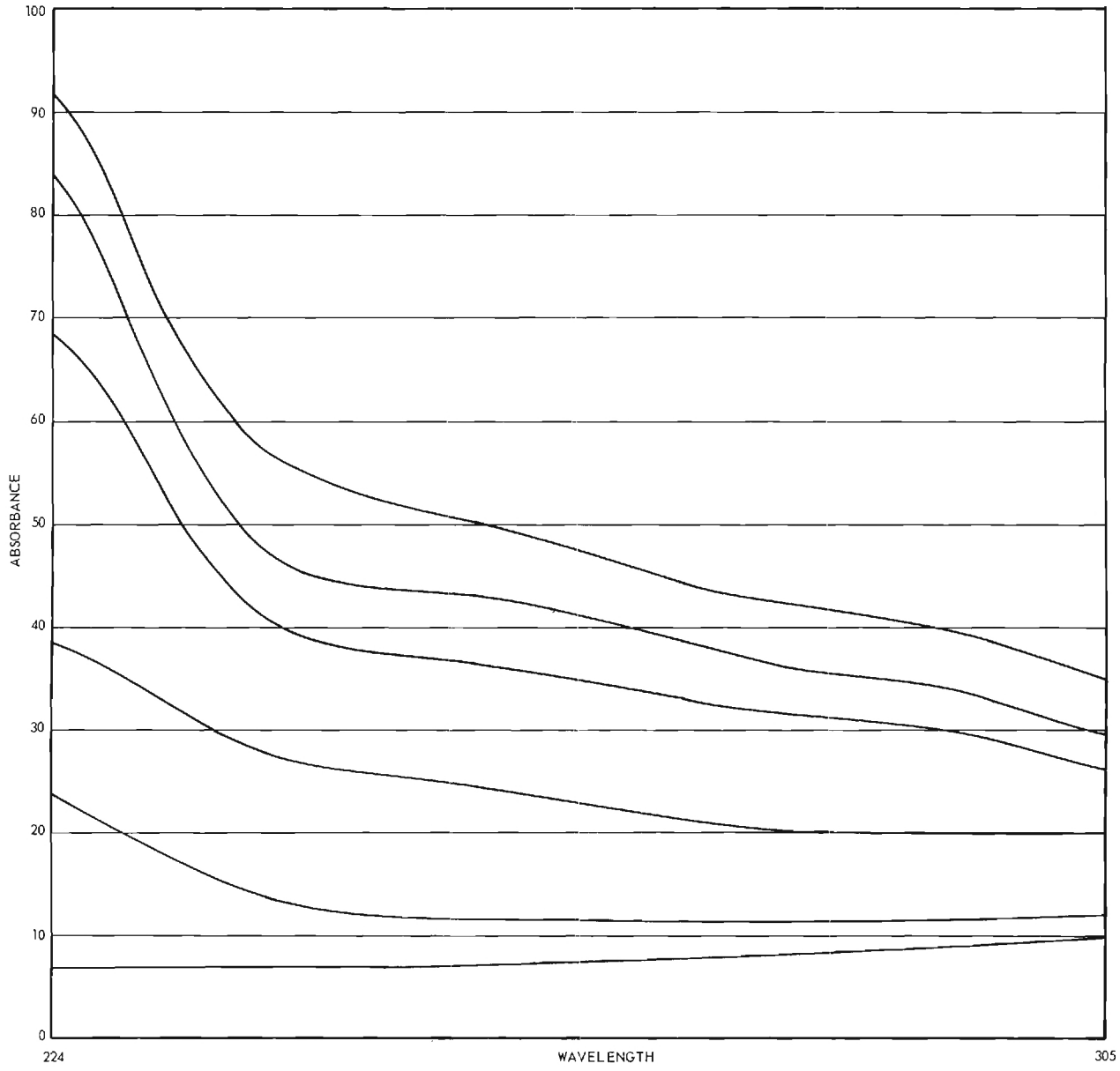

Figure 1. Typical analysis of irradiated ferrous ammonium sulfate solution at 305 mμ and 224 mμ. Beckman Model DK-2 Ratio recording spectrophometer.

III. RESULTS

A. YIELD OF INTERSTITIAL CHROMATID DELETIONS AS A FUNCTION OF RADIATION DOSE AT ROOM TEMPERATURE.

Table I presents the data obtained on unfrozen cell cultures irradiated with 50 r.

Table I

△ Time After Irradiation (hours)	No. Chromosomes (N)	No. Deletions (D)	$\frac{D}{N} \times 10^{-3}$
12	4016	9	2.2 ± 0.7
24	2642	0	-
36	2000	0	-
48	2000	0	-
60	2000	0	-

Table I. Number of deletions after various times with 50 r - unfrozen group.

Nine deletions were observed in 4,016 chromosomes at 12 hours after irradiation. At 24, 36, 48 and 60 hours after irradiation no deletions were observed after counting 2,000 chromosomes. This indicates that there was no mitotic inhibition induced by the irradiation at this dose level or that the mitotic inhibition did not persist longer than 12 hours, otherwise, some deletions would have been evident in the 24-hour group. In Table II the results of irradiation with 100 r in the unfrozen group are presented.

Table II

Δ Time After Irradiation (hours)	No. Chromosomes (N)	No. Deletions (D)	$\frac{D}{N} \times 10^{-3}$
12	1827	8	4.4 ± 1.5
24	3400	0	-
36	2000	0	-
48	2000	0	-
60	2000	0	-

Table II. Number of deletions after various times with 100 r - unfrozen group.

Eight deletions were observed in 1,827 chromosomes 12 hours after irradiation. At 24, 36, 48 and 60 hours after irradiation there were no deletions observed with counts of at least 2,000 chromosomes. Table III gives the results of 200 r irradiation.

Table III

Δ Time After Irradiation (hours)	No. Chromosomes (N)	No. Deletions (D)	$\frac{D}{N} \times 10^{-3}$
12	991	12	12.1 ± 3.5
24	415	0	-
36	369	0	-
48	400	0	-
60	400	0	-

Table III. Frequency of deletions at various time intervals with 200 r - unfrozen group.

With 200 r in the unfrozen cell cultures, 12 deletions were observed in 991 chromosomes and, as with the 50 and 100 r experiments, no deletions were observed 24, 36, 48 and 60 hours after irradiation. Table IV summarizes the results of 400 r in the unfrozen group.

Table IV

Δ Time After Irradiation (hours)	No. Chromosomes (N)	No. Deletions (D)	$\frac{D}{N} \times 10^{-3}$
12	-	-	-
24	613	10	16.0 ± 5
36	915	6	6.5 ± 2.6
48	772	4	5.2 ± 2.5
60	-	-	-

Table IV. Number of deletions after various times with 400 r - unfrozen group.

At 12 hours after irradiation no metaphases were observed, indicating there was a mitotic inhibition which prevented cells from entering metaphase 12 hours after this dose. Twenty-four hours after irradiation there were 10 deletions observed in 613 chromosomes. At 36 hours there were 6 deletions in 915 chromosomes, and at 48 hours there were 4 deletions in 772 chromosomes. These data indicate that with 400 r irradiation in the unfrozen group a mitotic inhibition is induced which lasts longer than 12 hours and which apparently persists for at least 48 hours after irradiation in some cells. There was a peak deletion production at 24 hours after irradiation.

The effect of 600 r in the unfrozen group is presented in Table V.

Table V

Δ Time After Irradiation (hours)	No. Chromosomes (N)	No. Deletions (D)	$\frac{D}{N} \times 10^{-3}$
12	-	-	-
24	621	15	24.0 ± 6
36	666	8	12.0 ± 4.2
48	-	-	-
60	-	-	-

Table V. Number of deletions after various times with 600 r - unfrozen group.

Again there were no metaphases observed at 12 hours after irradiation. At 24 hours there were 15 deletions at 621 chromosomes. At 36 hours there were 8 deletions in 666 chromosomes.

Table VI presents the results of 800 r in the unfrozen group.

Table VI

Δ Time After Irradiation (hours)	No. Chromosomes (N)	No. Deletions (D)	$\frac{D}{N} \times 10^{-3}$
12	-	-	-
24	416	14	33.6 ± 9.0
36	670	6	8.9 ± 3.5
48	-	-	-
60	-	-	-

Table VI. Number of deletions after various times with 800 r - unfrozen group.

Deletions were observed 24 hours after irradiation where there were 14 deletions in 416 chromosomes. Thirty-six hours after irradiation there were 6 deletions observed in 670 chromosomes.

The results of 1000 r are presented in Table VII.

Table VII

Δ Time After Irradiation (hours)	No. Chromosomes (N)	No. Deletions (D)	$\frac{D}{N} \times 10^{-3}$
12	-	-	-
24	420	17	40.4 ± 10
36	800	9	11.2 ± 4
48	-	-	-
60	-	-	-

Table VII. Number of deletions after various times with 1000 r - unfrozen group.

With 1000 r in the unfrozen group, 17 deletions were observed in 420 chromosomes 24 hours after irradiation. At 36 hours 9 deletions were observed in 800 chromosomes.

The information obtained in the first seven tables is summarized in Table VIII.

Table VIII

Dose (r)	Frequency of Deletions/1000 Chromosomes after irradiation time				
	12 hr	24 hr	36 hr	48 hr	60 hr
50	2.2 ± 0.7	0	0	-	-
100	4.3 ± 1.5	0	0	-	-
200	12.0 ± 3.5	0	0	-	-
400	0	16.0 ± 5	6.5 ± 2.6	-	-
600	0	24.0 ± 6	12 ± 4.2	-	-
800	0	33.6 ± 9	8.9 ± 3.5	-	-
1000	0	40.4 ± 10	11.2 ± 4	-	-

Table VIII. Summary of data on frequency of deletions after irradiation in unfrozen group.

The threshold dose for mitotic inhibition, which persists longer than 12 hours, is between 200 and 400 r. From 400 to 1000 r the peak chromatid production occurs 24 hours after irradiation. The production of deletions drops off very sharply after 24 hours. A scatter plot of the deletion frequency as a function of irradiation dose in the unfrozen group is presented in Figure 2. The estimating equation determined by the method of the least squares was $Y = 1.378 + .039X$. There was a high positive linear correlation between interstitial chromatid deletions and radiation dose. There were 4×10^{-5} interstitial deletions/chromosomes/r.

Under the conditions of these experiments a mitotic inhibition was produced in the unfrozen cell cultures between doses of 200 - 400 r which persisted for longer than 12 hours.

B. YIELD OF INTERSTITIAL CHROMATID DELETIONS AS A FUNCTION OF RADIATION DOSE AT -190°C (LIQUID NITROGEN).

Table IX presents the data on frequency of deletions produced with 75 r in the group irradiated in liquid nitrogen.

Table IX

Δ Time After Irradiation (hours)	No. Chromosomes (N)	No. Deletions (D)	$\frac{D}{N} \times 10^{-3}$
12	6131	6	0.98 ± 0.4
24	4000	0	-
36	-	-	-
48	-	-	-
60	-	-	-

Table IX. Number of deletions observed after various times with 75 r - frozen group.

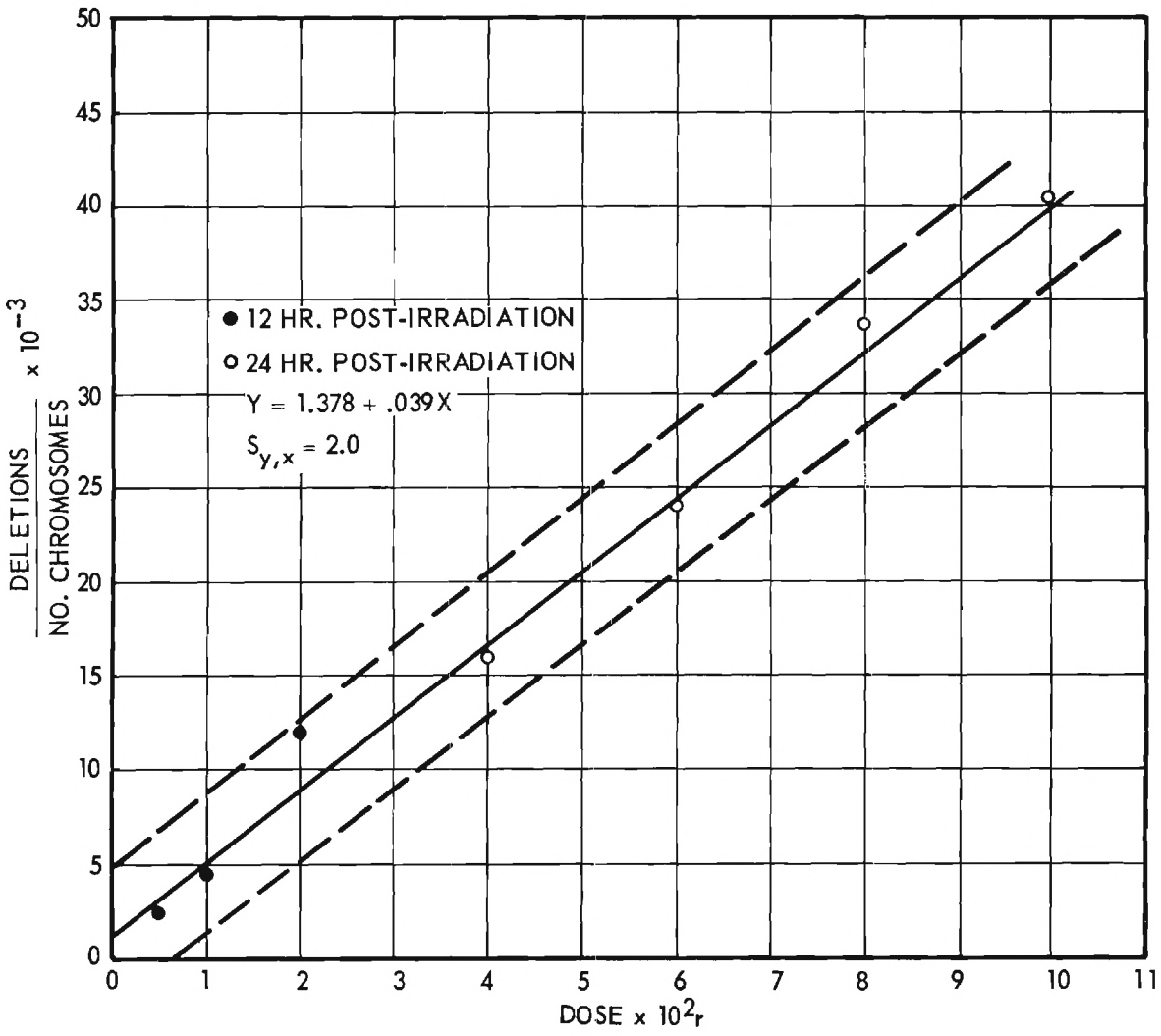

Figure 2. Maximum number of chromatid deletions produced at various time increments after irradiation as a function of x-ray dose. Irradiation performed at room temperature.

Twelve hours after irradiation there were 6 deletions in 6,131 chromosomes. There were no deletions observed in 4,000 chromosomes 24 hours after irradiation. The results of 300 r irradiations are presented in Table X .

Table X

Δ Time After Irradiation (hours)	No. Chromosomes (N)	No. Deletions (D)	$\frac{D}{N} \times 10^{-3}$
12	2464	10	4.0 ± 1.3
24	653	0	-
36	-	-	-
48	-	-	-
60	-	-	-

Table X . Number of deletions observed after various times with 300 r - unfrozen group.

With 300 r there were 10 deletions produced in 2,464 chromosomes and no deletions observed in 653 chromosomes 24 hours after irradiation. Table XI gives the results with 800 r.

Table XI

Δ Time After Irradiation (hours)	No. Chromosomes (N)	No. Deletions (D)	$\frac{D}{N} \times 10^{-3}$
12	1062	11	10.3 ± 3.2
24	523	0	-
36	-	-	-
48	-	-	-
60	-	-	-

Table XI . Number of deletions observed after various times with 800 r - frozen group.

There were 11 deletions in 1,062 chromosomes 12 hours after irradiation and, at 24 hours, there were no deletions observed in 523 chromosomes. With 1200 r, Table XII , shows there were 17 deletions in 1,087 chromosomes 12 hours after irradiation, and 24 hours after irradiation there were no deletions observed in 620 chromosomes.

Table XII

Δ Time After Irradiation (hours)	No. Chromosomes (N)	No. Deletions (D)	$\frac{D}{N} \times 10^{-3}$
12	1087	17	15.7 ± 3.8
24	620	0	-
36	-	-	-
48	-	-	-
60	-	-	-

Table XII . Number of deletions observed after various times with 1200 r - frozen group.

In TableXIII the results of 2000 r show that there were 15 deletions recorded in 587 chromosomes 12 hours after irradiation. One deletion was observed in 625 chromosomes 24 hours after irradiation and no deletions were observed in 500 chromosomes at 36 hours after irradiation.

Table XIII

Δ Time After Irradiation (hours)	No. Chromosomes (N)	No. Deletions (D)	$\frac{D}{N} \times 10^{-3}$
12	587	15	25.6 ± 6.6
24	625	1	1.6
36	500	0	-
48	-	-	-
60	-	-	-

Table XIII. Number of deletions observed various times after 2000 r - frozen group.

The data on irradiation in the frozen group is summarized in Table XIV. With irradiation up to 2000 r there was no mitotic inhibition detected as was evident between 200 and 400 r in the unfrozen group.

Table XIV

Dose (r)	Frequency of Deletions/1000 Chromosomes after irradiation time				
	12 hr	24 hr	36 hr	48 hr	60 hr
75	0.98 ± 0.4	0	-	-	-
300	4.0 ± 1.3	0	-	-	-
800	10.3 ± 3.2	0	-	-	-
1200	15.7 ± 3.8	0	-	-	-
2000	25.6 ± 6.6	1.6	0	-	-

Table XIV. Summary of data on frequency deletions noticed after irradiation in frozen group.

Figure 3 is a diagram of the frequency of chromatid deletions as a function of radiation dose. The points used represent the maximum frequency of deletions produced after irradiation. In the frozen group the maximum chromatid production was 12 hours after irradiation of all doses. The estimating equation determined on the method of the least squares was $Y = .816 + .012X$. There was a high positive linear correlation between interstitial chromatid deletions and radiation dose. There were 1.3×10^{-5} interstitial deletion/chromosome/r.

Cells irradiated in liquid nitrogen with doses to 2000 r did not exhibit a mitotic inhibition which lasted as long as 12 hours.

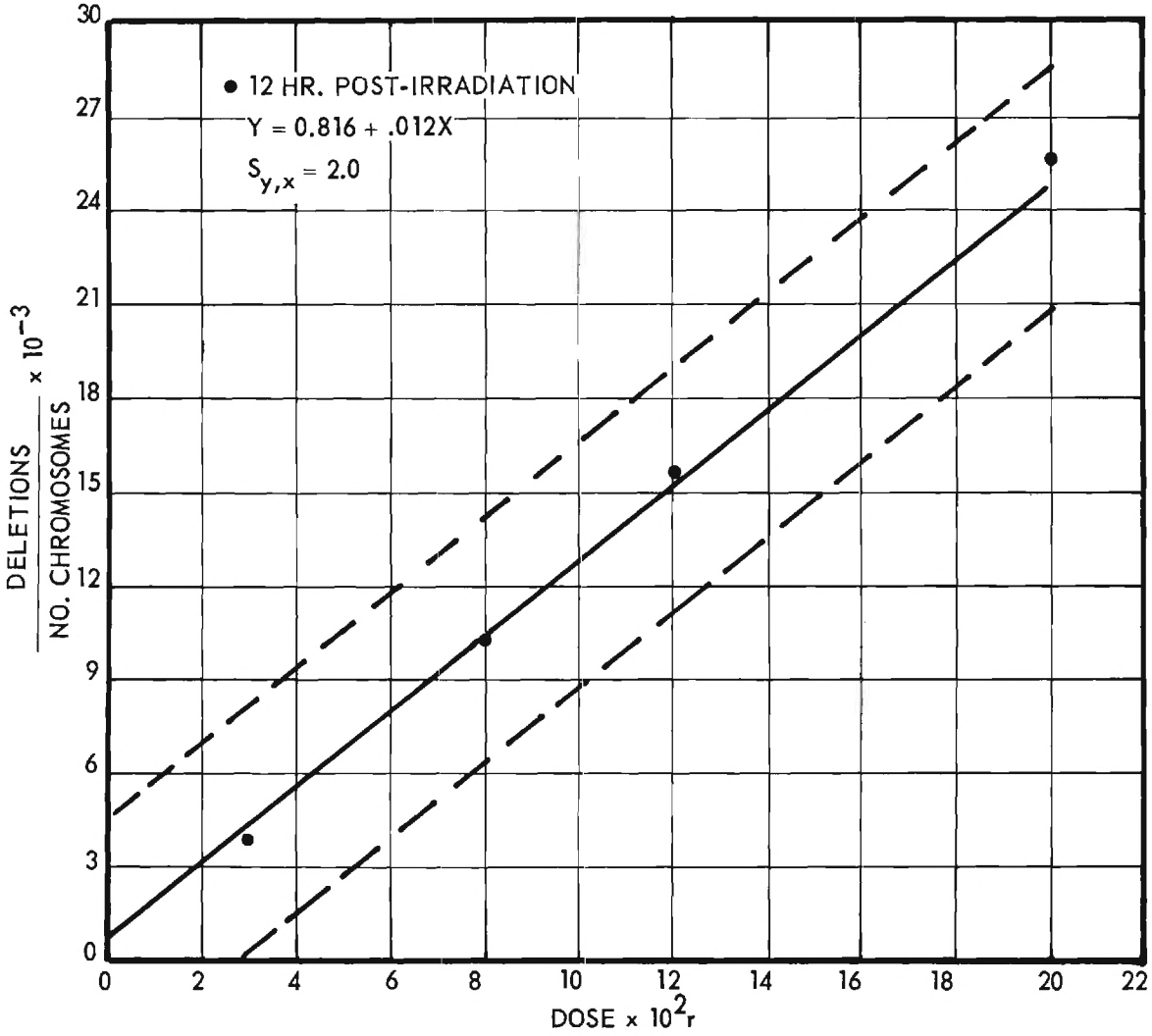

Figure 3. Maximum number of chromatid deletions produced a at various time increments after irradiation as a function of x-ray dose. Irradiation performed with cells in liquid nitrogen (-190°C).

C. YIELD OF INTERSTITIAL CHROMATID DELETIONS WITH 1000 r AT -190°C WITH INCREMENTS OF 0.5, 1, 2, 4, 8 AND 30 DAYS SEPARATION OF TWO EQUAL DOSES OF 500 r.

The results of the dose fractionation experiments are presented in Table XV. There was not a significant difference between the dose fractionation yield of frequency of deletions and the previously determined figure for deletions per 1000 r which was equal to 12.8 X 10^{-3} deletion/chromosome. Over this period of time there is apparently no effect of dose fractionation on yield of interstitial chromatid deletions.

Table XV

Time between dose fractions (days)	Number of Chromosomes	Frequency of Interstitial Chromatid Deletions X 10^{-3}	"t" Table level of significance
0*	-	12.8 ± 0.8	-
0.5	1019	12.2	.60
1.0	818	13.4	.60
2.0	971	13.7	.50
4.0	1011	12.1	.50
8.0	853	13.9	.40
30.0	975	12.4	.60

*Single dose of 1000 r (see Progress Report No. 3) frequency determined from estimating equation; freq. = .816 ± .012 dose.

Table XV. Frequency of interstitial chromatid deletions produced by two doses of 500 r of 250 KV X-rays separated by various increments of time.

D. YIELD OF INTERSTITIAL CHROMATID DELETIONS WITH 1000 r AT -190°C WITH IRRADIATION INTENSITIES OF 100, 10 AND 1 r/MIN.

Table XVI indicates the yield of chromatid deletions with various radiation intensities.

TABLE XVI

Dose Intensity (r/min)	Total Dose (r)	No. Chromosomes C	No. Deletions D	Frequency $\frac{D}{N} \times 10^{-3}$
100	1000	2033	26	12.8
10	1000	905	11	12.2
1	1000	2164	29	13.4

Over this range of dose intensities there is apparently no difference in the yield of chromatid deletions. Radiation of very low intensities (1 r/min) is difficult to calibrate with the X-ray equipment used.

E. Fe^{+++} ION YIELD AS A FUNCTION OF RADIATION DOSE AT ROOM TEMPERATURE AND AT -190°C.

A series of experiments was run in which the Fricke dosimeter was irradiated in both the frozen and unfrozen state. The results are presented in Figure 4. Irradiation of the ferrous ammonium sulfate solution in liquid nitrogen results in approximately one-fourth the yield of ferric ions that is produced by irradiation at room temperature. The yield, as a function of dose, did not show as good a fit to a straight line in the frozen state as is characteristic of this dosimeter in the unfrozen condition, so additional quantitation will be needed.

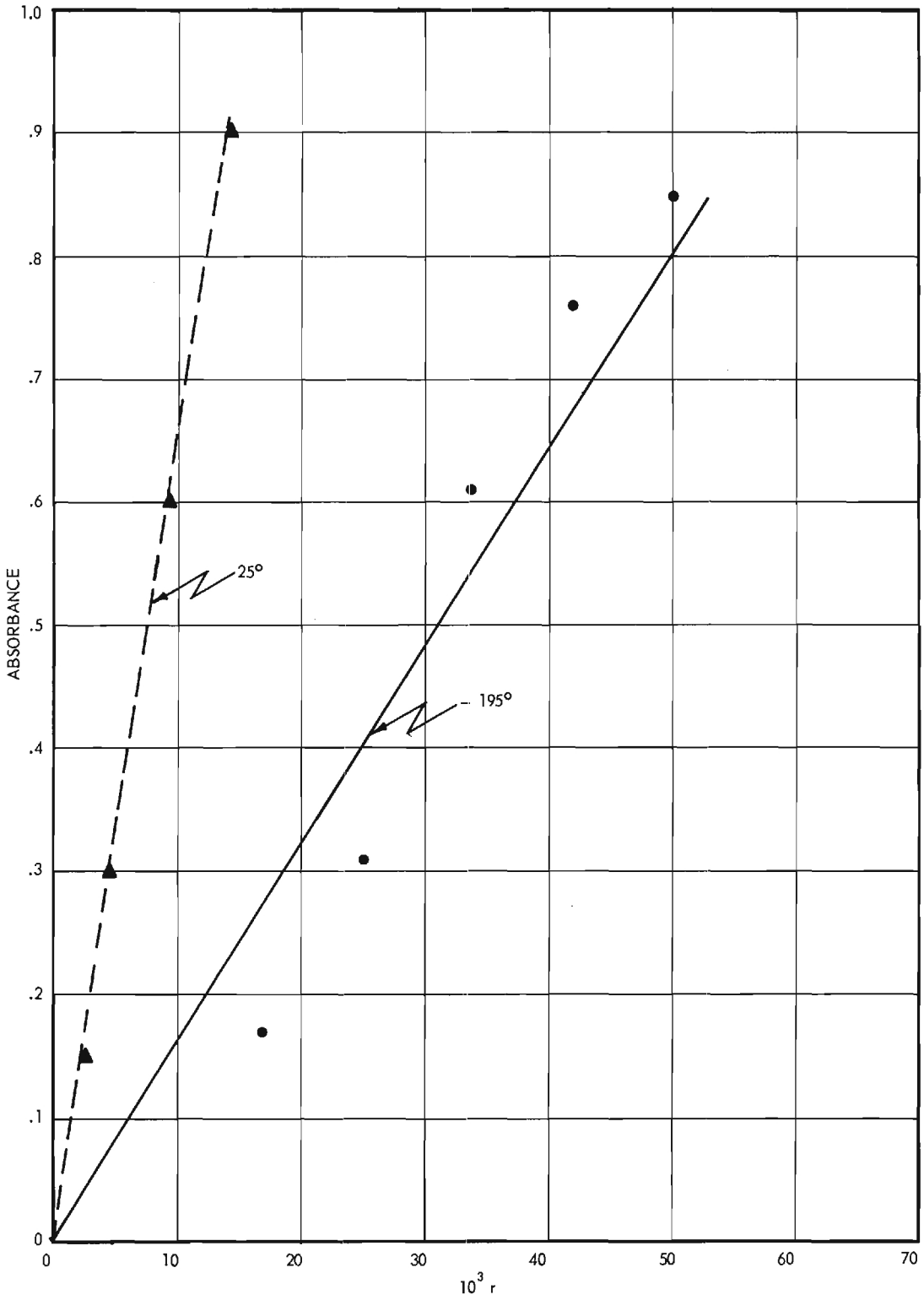

Figure 2. Optical density (224 mμ) of irradiated ferrous ammonium sulfate solution as a function of X-ray dose.

F. USE OF 10 cm CELL FOR Fe^{+++} DETERMINATION.

In Table XVII the results of irradiation experiments performed on several different days are presented. There is a high positive correlation between dose and absorbance, indicating a good reproducibility of this experimental procedure and also indicating that increasing the path length to 10 centimeters extends the useful range of this chemical dosimeter down into the range of greatest biological interest. At the point of greatest sensitivity of the spectrophotometer, 0.3 optical density (50% transmission), the dose is equal to 500 ± 10 r, indicating a 2% variance.

Table XVII

Absorbance (Optical Density)	Dose (r)	Theoretical dose*
.08	125	117
.11	130	162
.16	250	232
.24	375	353
.23	400	338
.33	500	500
.33	500	500
.42	625	617
.44	650	647
.50	750	735
.53	780	780
.60	875	880
.60	910	880
.70	1000	1030
.67	1000	985
.68	1170	1200
.82	1170	1200

*Absorbance x 1470 = dose

Least squares analysis: absorbance = 1.70 + .069 dose $S_{dose,\ absorbance}$ = 10

Table XVII. Absorbance of Fe^{+++} ions as a function of radiation dose in a 10 cm absorption cell.

10 ml ampules were filled with ferrous ammonium sulfate solution and heat-sealed. The ampules were frozen and irradiated submerged in liquid nitrogen in open-top DeWar flasks. After irradiation the ampules were thawed and the concentration of ferric ion measured in the spectrophotometer. The results of several of these experiments is presented in Table XVIII.

Table XVIII

Absorbance (Optical Density)	Dose (r)	Theoretical dose*
.04	100	59
.09	240	132
.09	400	132
.18	520	262
.12	520	176
.20	650	294
.26	780	382
.20	780	294
.30	920	440
.32	1020	470
.40	1180	587

*Absorbance x 1470 = dose in r

Table XVIII. Absorbance of Fe^{+++} ions as a function of radiation dose in a 10 cm absorption cell. Solutions irradiated at -190°C.

In all cases the yield of ferric ions as determined by absorbance at 224 mμ was less when irradiation was performed at liquid nitrogen temperatures than at room temperature. The yield of ferric ions as a function of radiation dose in these experiments, however, was not reproducible from experiment to experiment, and even within an experimental group. The yield did not appear

to be linear with dose. It may be that a number of factors could influence the yield of ferric ions, including rate of freezing and rate of thawing. It was brought to our attention also that there is a tendency to freeze out dissolved materials during the formation of ice crystals. In this case there would be an actual freezing out of the ferrous ions and an unequal concentration of them in the solution.

G. EFFECT OF GLYCERINE ON YIELD OF Fe^{+++} IONS.

The effects of the addition of glycerol was examined in a preliminary investigation. Insofar as glycerine is used in freezing of the biological dosimeter, a preliminary investigation seemed worthwhile. It is known that the addition of organic materials to the Fricke dosimeter results in an increased yield with considerable evidence to suggest that as many as 3 ferric ions can be reoxidized as the result of an organic intermediate radical and the justification for the addition of sodium chloride to the ferrous ammonium sulfate solution is the result of a demonstration that it suppresses the effect of organic contaminants through a differential oxidation of the chloride. We, however, wished to investigate the effect of the glycerine primarily in the frozen state because of its known effect on ice crystal formation. The preliminary results of these experiments are presented in Table XVIII. The presence of the glycerine precluded the use of the 10 centimeter cells, there being insufficient energy in our spectrophotometer to traverse the glycerine solution. Experiments were performed at higher dose rates using a 1 centimeter path length cell. The results are presented in Table XIX and it can be seen that there was no improvement in reproducibility either between replicate samples or between experimental points determined on separate days. It would

appear that the addition of glycerine did not markedly increase yield in the frozen state and it did not increase the reproducibility.

It would appear that without additional work involving extensive experimentation on the rates of freezing, rates of thawing and the effect of other factors on the frozen dosimeter that the system is not reproducible enough to yield useful information pertinent to an understanding of events transpiring in the biological dosimeter.

Table XIX

Absorbance (Optical Density)	Dose (r)	Theoretical dose*
.58	5000	8232
.64	5000	9408
.70	7500	1029
.64	7500	9408
.91	10,000	13,230
.82	10,000	12,056
.19	5000	2800
.13	5000	1900
.26	7500	3820
.22	7500	3230
.33	10,000	5000
.26	10,000	3800

*Absorbance x 14700 = dose in r

Table XIX. Absorbance of Fe^{+++} ions as a function of radiation dose in a 1 cm absorption cell. Solutions contained 10% (volume/volume) glycerol.

IV. DISCUSSION

The reduction in yield of chromosome breakage as a function of radiation dose in frozen cells reported in these data is different from that reported by Fabergé. Our data indicates a three-fold decrease in sensitivity in the frozen state as compared with a five-fold (1950) and two-fold (1954) decrease from the reports of Fabergé, although different biological systems are involved -- the work of Fabergé having been performed with Tradescantia pollen. These reports appear to be the only ones available on the production of chromosome breakage at very low temperatures. Other biological and chemical systems have been studied, however, and the work with bacteria, for example, indicates that the irradiation sensitivity decreases to about 1/3 between room temperature and liquid air temperature for E. coli. This same magnitude of sensitivity reduction appears to be characteristic for the inactivation of dry bacteriophage, dry catalase, the breakage of carbon to carbon bonds in polyisobutylenes, and irradiation of dry spores of Bacillus megaterium.

The demonstration that a mitotic inhibition was induced which lasted for longer than 12 hours after irradiation with 200 to 400 r in the unfrozen cell cultures is in general agreement with other reports. Radiation-induced mitotic inhibition is known to have a threshold value and the duration of the inhibition has been demonstrated to be dose dependent. The failure of cells in the frozen state to demonstrate a mitotic inhibition even at very high doses is significant. The exact nature of the mitotic inhibition is unknown but the presence of a threshold value (multihit effect) has been interpreted as evidence that it may be the result of an interference with some metabolic process. The protection which the frozen state provides, as evidenced in

these data, may indicate that the mitotic inhibition is a result of events which take place in that part of the cell rendered inactive (water?) by very low temperatures.

The results of dose fractionation experiments and dose intensity experiments indicate that the yield of interstitial chromatid deletions as a function of radiation dose is independent of both fractionation and dose intensity over a wide dose range. Thus frozen human cells may provide a useful dosimeter which can accumulate radiation over long periods of time.

The Fricke chemical dosimeter failed to give reproducible yields of Fe^{+++} ions when irradiated in the frozen state.

V. FINANCIAL REPORT

For the period July 1, 1961 through June 30, 1963 the following expenditures were made:

Personnel Services	$27,520.44
Materials and Supplies	2,545.42
Equipment	138.50
Freight and Express	40.24
Overhead	17,888.29
TOTAL	$48,132.89

Respectfully submitted:

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Approved:

W. B. Harrison, Chief
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APPENDIX

Table XX

Computation of sums for correlation of 250 KV X-ray dose (X) and deletions observed (Y) in chromosomes of KB cells 12 hours after irradiation

X	Y	X^2	Y^2	XY
75	0.98	5,625	0.96	73.4
300	4.0	90,000	16.00	1,200.0
800	10.3	640,000	106.09	8,240.0
1200	15.7	1,440,000	246.49	18,840.0
2000	25.6	4,000,000	655.36	51,200.0
4375	56.58	6,175,625	1,024.90	79,553.4

I. $\Sigma Y = Na + b\Sigma X$

II. $\Sigma XY = z\Sigma X + bX^2$

I. $56.58 = 5a + 4375b$

II. $79553.4 = 4375a + 6,175,625b$

$79553.4 = 4375a + 6,175,625b$

$49507.5 =$

$30045.9 =$

$b =$

$a =$

$Y = .816 +$

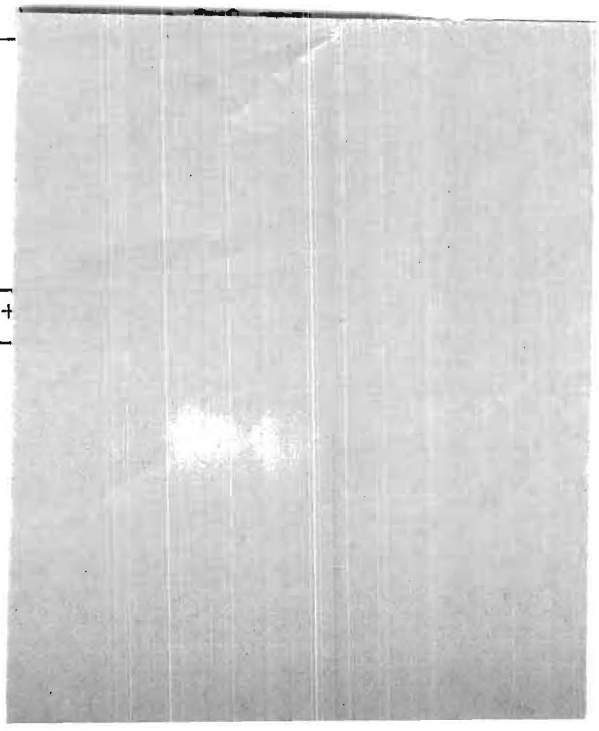

Table XXI

Computation of sums for correlation of roentgens of 250 KV X-rays (X) and deletions observed (Y) in chromosomes of KB cells 12 and 24 hours after irradiation

X	Y	X^2	Y^2	XY
50	2.2*	2,500	4.84	110
100	4.3*	10,000	18.49	430
200	12.0*	40,000	144.00	2,400
400	16.0**	160,000	256.00	6,400
600	24.0**	360,000	576.00	14,400
800	33.6**	640,000	1,128.96	26,880
1000	40.4**	1,000,000	1,632.16	40,400
3150	132.5	2,212,500	3,760.45	91,020

* 12 hours after irradiation

** 24 hours after irradiation

I. $\Sigma Y = Na + b\Sigma X$

II. $\Sigma XY = a\Sigma X + bX^2$

I. $132.5 = 7a + 3150b$

II. $91,020 = 3150a + 2,212,500b$

$91,020 = 3150a \quad 2,212,500b$

$59,625 = 3150a \quad 1,417,500b$

$31,405 = \quad 795,000b$

$b = .039$

$a = 1.378$

$$Y = 1.378 + .039X$$